Nucleic acids

Nucleic acids

- Nucleic acids was first discovered by **Friedrich Miescher** from the nuclei of the pus cells (**Leukocytes**) from the discarded surgical bandages and called it **Nuclein**
- Nuclein was later shown to be a mixture of a basic protein and a phosphorus- containing organic acid, now called nucleic acid
- There are 2 types of nucleic acids (polynucleotides) – **Ribonucleic Acid** (**RNA**)

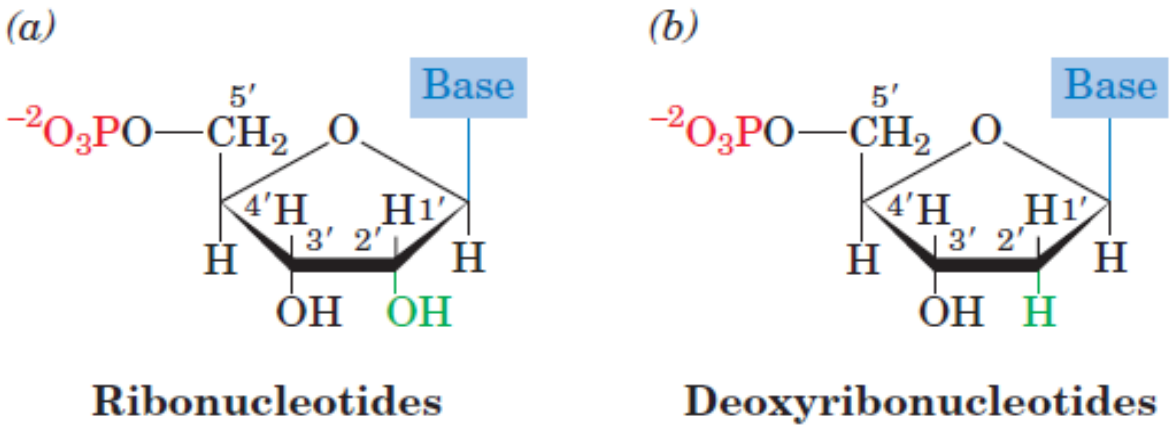
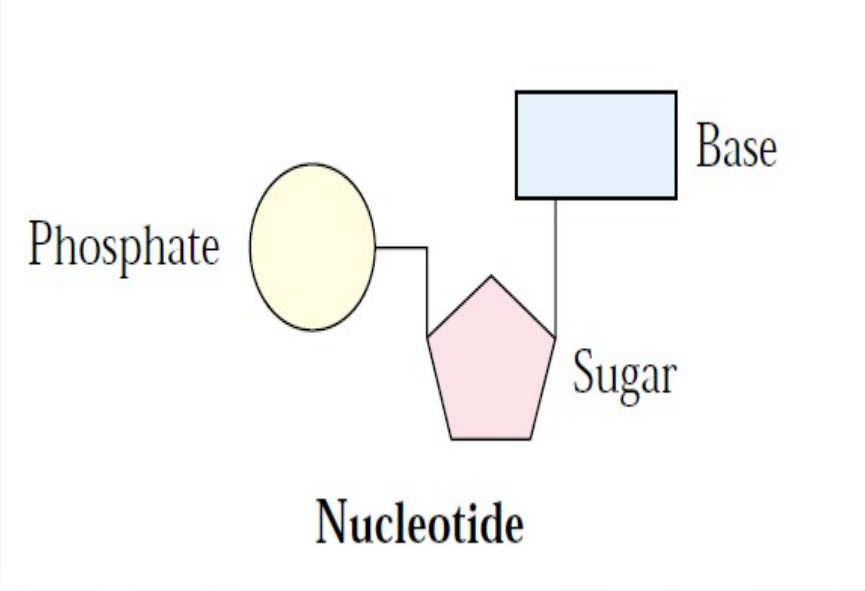
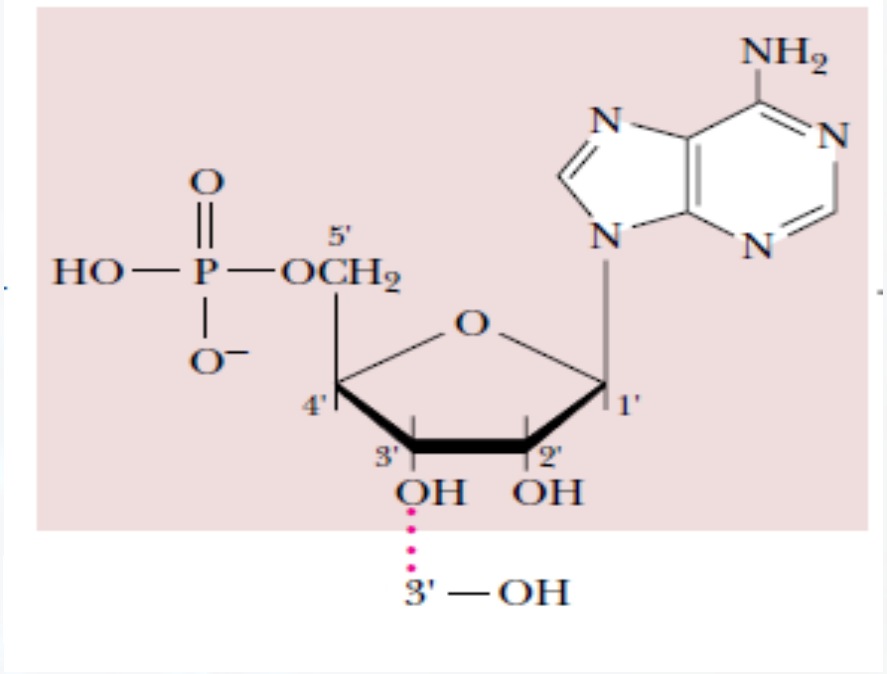


Figure 5-1 **Chemical structures of (*a*) ribonucleotides and (*b*) deoxyribonucleotides.**

Nucleotides

- The monomeric units of nucleic acids are called **Nucleotides**
- Nucleic acids therefore are also called **Polynucleotides**
- Nucleotides are **Phosphate Esters Of Nucleosides** and made up of 3 components-
  - **A base that has nitrogen atom (nitrogenous base)**
  - **A 5 carbon sugar**
  - **An ion of phosphoric acid**





Nitrogenous bases

Are heterocyclic, planar and relatively water insoluble aromatic molecules.

There are 2 general types of nitrogenous bases in both RNA and DNA:

1. **Pyrimidines**
2. **Purines**

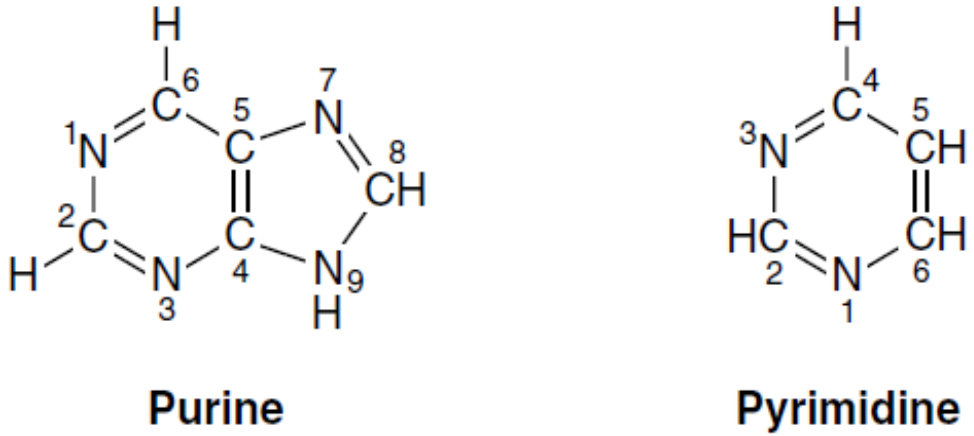
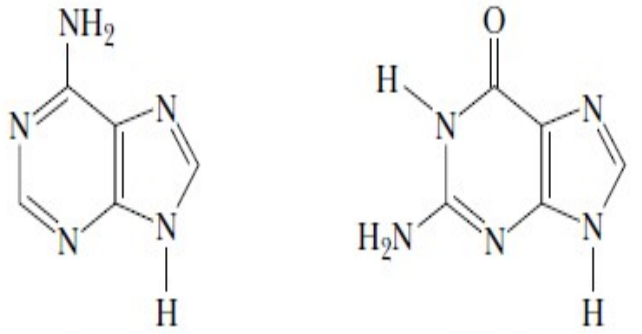


Figure 33–1. Purine and pyrimidine. The atoms are numbered according to the international system.

Purines

- 2 different nitrogenous bases with a purine ring (composed of carbon and nitrogen) are found in DNA.
- The 2 common purine bases found in DNA and RNA are
  - **Adenine (6-aminopurine)**
  - **Guanine (6-oxy-2-aminopurine)**
- **Adenine** has an **Amino Group (-NH_2)** on the **C_6** position of the ring (carbon at position 6 of the ring)
- **Guanine** has an **Amino Group (-NH_2)** at **C_2** position and a **Carbonyl Group** at the **C_6** position



Adenine (6-amino purine)

Guanine (2-amino-6-oxy purine)

FIGURE 11.4 • The common purine bases—adenine and guanine—in the tautomeric forms predominant at pH 7.

Pyrimidines

- 2 major pyrimidine bases found,
- In DNA are,
  - **Thymine (2-oxy-4-oxy 5-methyl pyrimidine)**
  - **Cytosine (2-oxy-4-aminopyrimidine)**
- In RNA
  - **Uracil (2,4-dioxypyrimidine)**
- **Thymine** contains a **Methyl Group** at the C_5 position with **Carbonyl Groups** at the C_4 and C_2 positions
- **Cytosine** contains a **Hydrogen Atom** at the C_5 position and an **Amino Group** at C_4
- **Uracil** is similar to **Thymine** but lacks the **Methyl Group** at the C_5 position
- Uracil is not usually found in DNA. It is the component of RNA

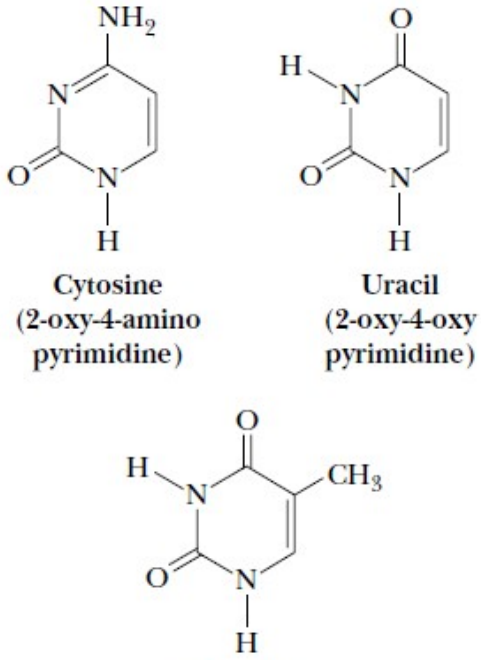
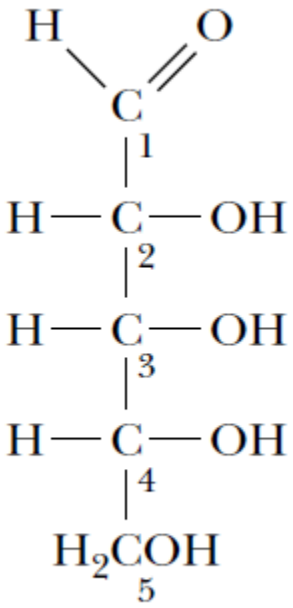


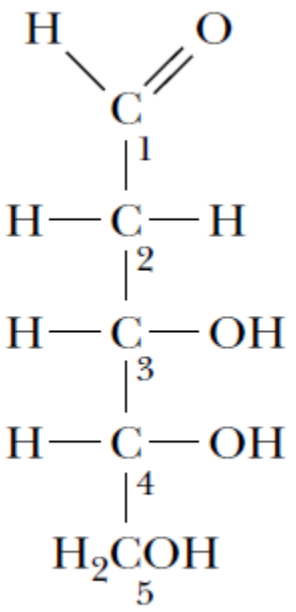
FIGURE 11.3 • The common pyrimidine bases—cytosine, uracil, and thymine—in the tautomeric forms predominant at pH 7.

Sugars

- Naturally occurring nucleic acids have 2 types of pentose sugars- **Ribose** and **Deoxyribose** sugars
- **Ribose** sugar is found in **RNA**
  - It is a **5 carbon monosaccharide with a hydroxyl group(-OH) on each carbon**
- **Deoxyribose** sugar is found in **DNA**
  - It is 5 carbon monosaccharide, **lacking one oxygen atom at 2' position**
- The **hydroxyl group (-OH)** at 2' position of ribose sugar is replaced by a **Hydrogen (-H)**
- The **Carbon Atoms** of the Ribose and Deoxyribose present in nucleoside or nucleotides are designated with a **prime (') mark**



D-Ribose



D-2-Deoxyribose

Nucleoside

- **Sugar + Nitrogenous Base = Nucleoside**
- The bond between the sugar and the base is called **N- glycosidic bond**
- The nitrogenous base lies above the plane of the sugar when the structure is written in the standard orientation. That is the configuration of the N-glycosidic linkage is **β**
- The base is free to rotate around the glycosidic bond. Due to rotation of the glycosidic bond, two different conformations are possible
- The 2 standard conformations of the base around the glycosidic bond are **Syn** and **Anti**
- Pyrimidines tend to adopt the anti conformation almost exclusively, because of steric interference between O_2 and C_5' in the **syn** conformation, whereas purines are able to assume both forms

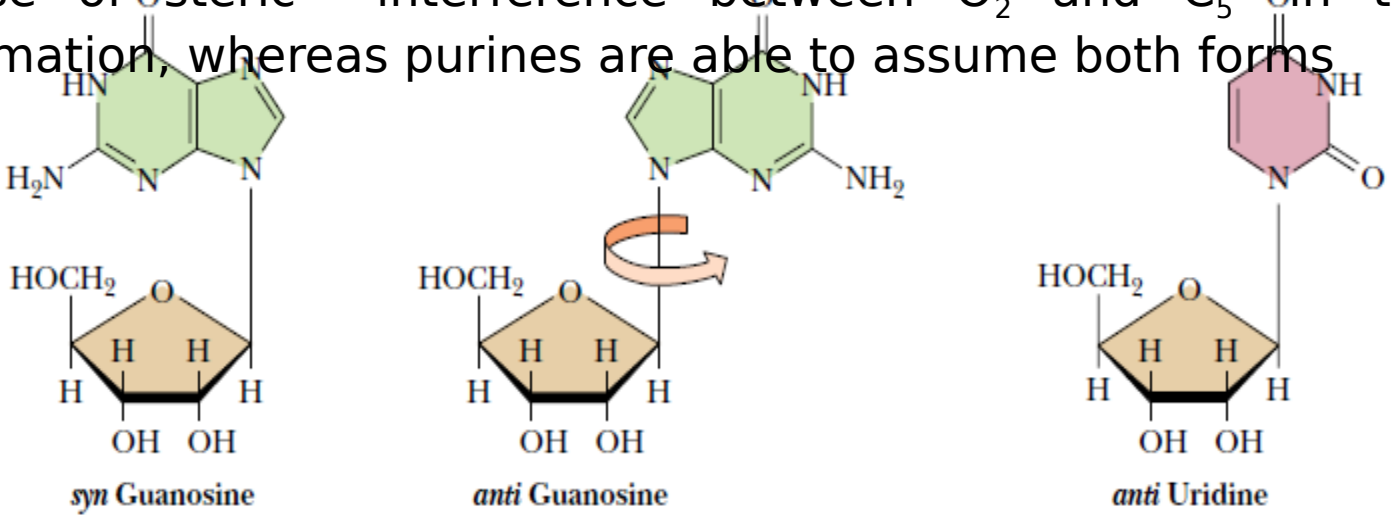


FIGURE 11.12 • Rotation around the glycosidic bond is sterically hindered; syn versus anti conformations in nucleosides are shown.

Functions of Nucleotides:

- Are primarily responsible for the formation of polynucleotides (nucleic acids of the cell- RNA and DNA)
- Polynucleotides are informational macromolecules. They store, transmit and translate genetic information
- However, they also are required for numerous other important functions within the cell
- These functions are-
- Formation of energy currency like ATP, GDP etc.
- Act as a precursor for several important coenzymes such as **NAD^+, $NADP^+$, FAD** and **coenzyme A**
- Serving as a precursor for

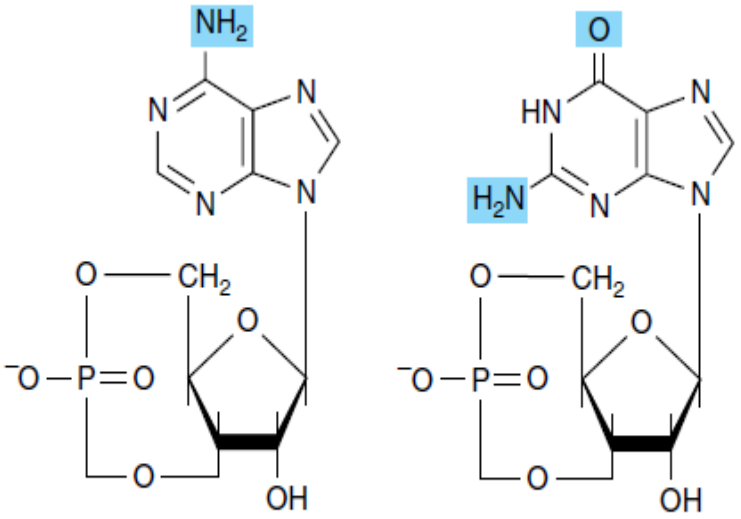


Figure 33–9. cAMP, 3′,5′-cyclic AMP, and cGMP.

- ATP is a chemical link between catabolism and anabolism
  - It is the energy currency of living cells
  - It acts as a donor of high energy phosphate
  - ATP consists of adenosine moiety to which **3 phosphoryl groups ($-PO_3^{2-}$)** are sequentially linked via **Phosphoester Bond** followed by **2 phosphoanhydride bonds**, referred to as a high energy bond
  - The active form of ATP is usually a complex of ATP with **Mg^{2+} / Mn^{2+}**

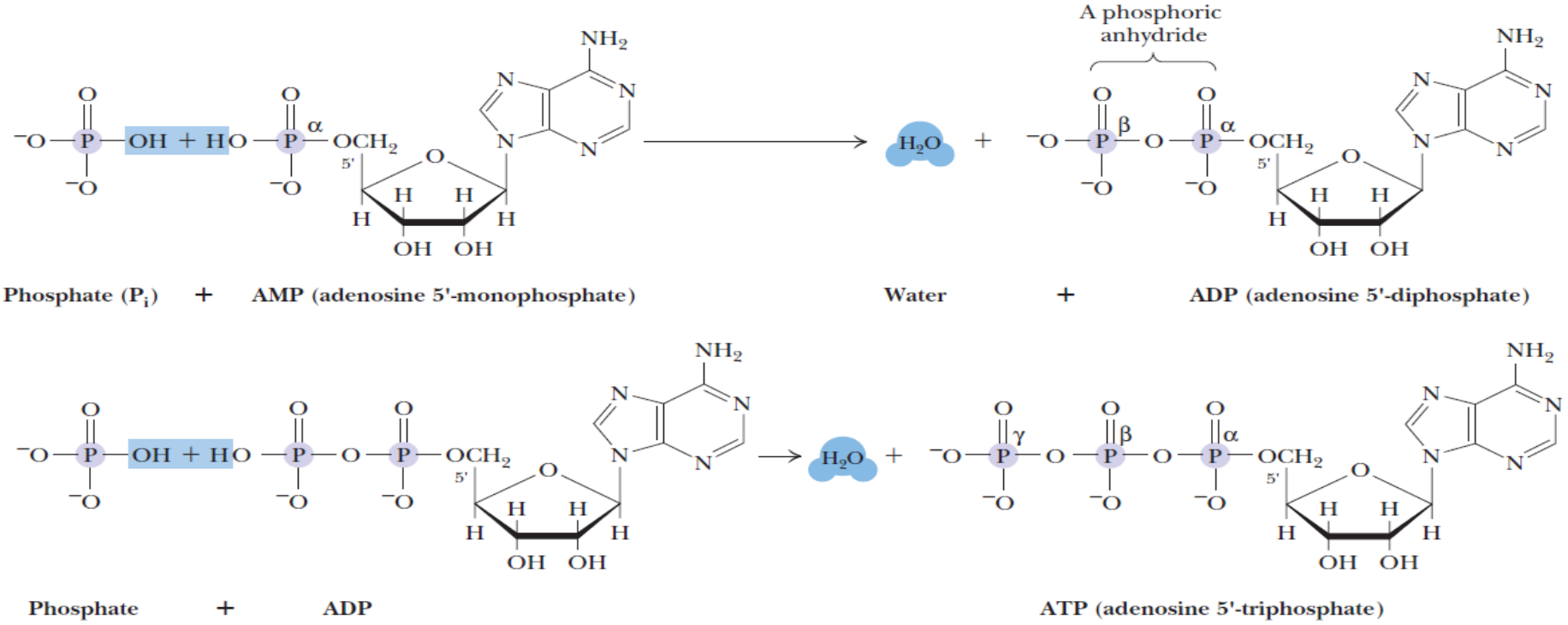

A phosphoric anhydride
Phosphate (P_i) + AMP (adenosine 5'-monophosphate)
Water + ADP (adenosine 5'-diphosphate)
Phosphate + ADP
ATP (adenosine 5'-triphosphate)

FIGURE 11.15 • Formation of ADP and ATP by the successive addition of phosphate groups via phosphoric anhydride linkages. Note the removal of equivalents of H_2O in these dehydration synthesis reactions.

Naming of nucleosides and nucleotides

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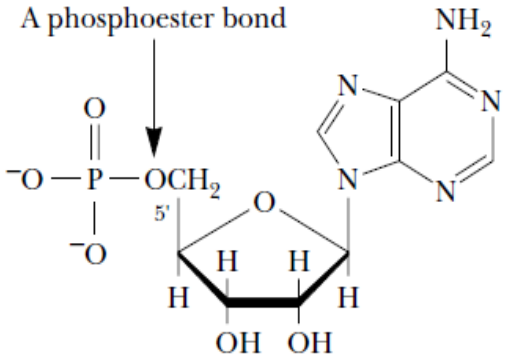
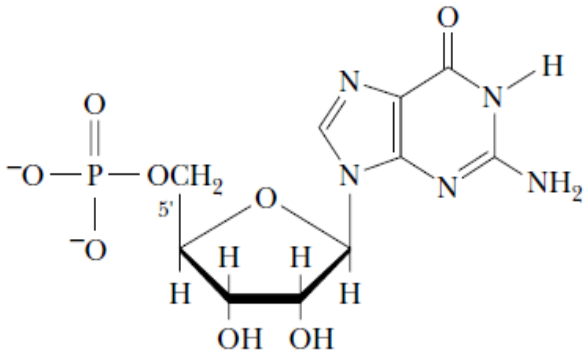
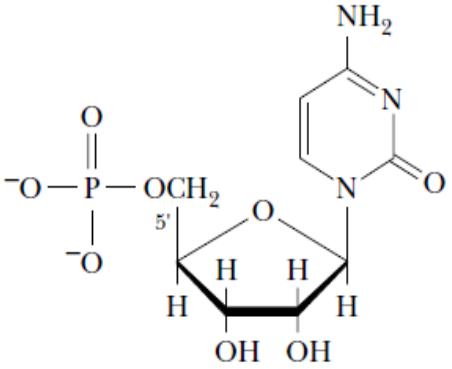
	Purines		Pyrimidines	
	Adenine(A)	**Guanine (G)**	**Cytosine (C)**	**Uracil(U) /thymine (T)**
Nucleoside RNA DNA	Adenosine deoxyadenosine	Guanosine deoxyguanosine	Cytidine deoxycytidine	Uridine deoxyuridine
Nucleotides in RNA DNA	Adenylate Deoxyadenylate	Guanylate Deoxyguanylate	Cytidylate Deoxycytidylate	Uridylate Deoxyuridylate
Nucleoside monophosphate	AMP	GMP	CMP	UMP/ TMP
Nucleoside diphosphate	ADP	GDP	CDP	UDP /TDP



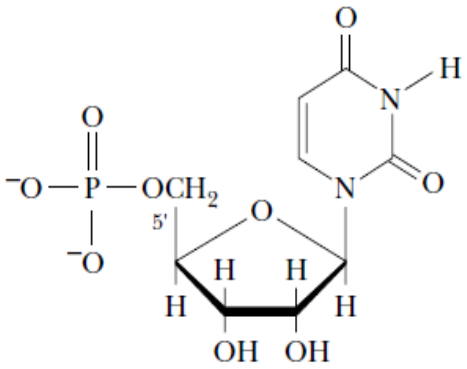
Adenosine 5'-monophosphate
(or AMP or adenylic acid)



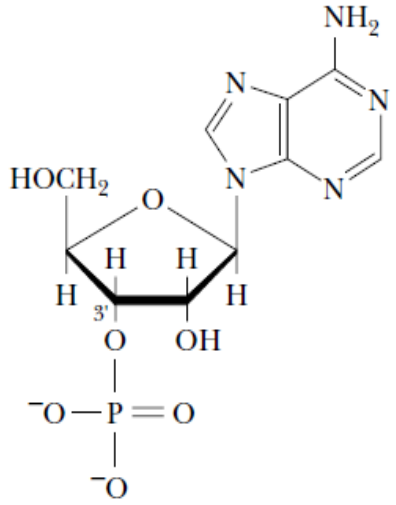
Guanosine 5'-monophosphate
(or GMP or guanylic acid)



Cytidine 5'-monophosphate
(or CMP or cytidylic acid)



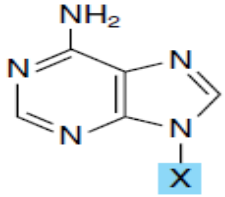
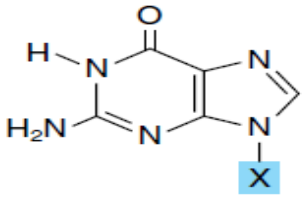
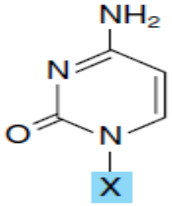
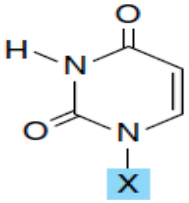
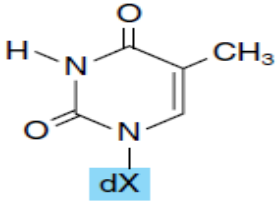
Uridine 5'-monophosphate
(or UMP or uridylic acid)



A nucleoside 3'-monophosphate
3'-AMP

FIGURE 11.13 • Structures of the four common ribonucleotides—AMP, GMP, CMP, and UMP—together with their two sets of full names, for example, adenosine 5′-monophosphate and adenylic acid. Also shown is the nucleoside 3′-AMP.

Table 33–1. Bases, nucleosides, and nucleotides.

Base Formula	Base X = H	Nucleoside X = Ribose or Deoxyribose	Nucleotide, Where X = Ribose Phosphate
	Adenine A	Adenosine A	Adenosine monophosphate AMP
	Guanine G	Guanosine G	Guanosine monophosphate GMP
	Cytosine C	Cytidine C	Cytidine monophosphate CMP
	Uracil U	Uridine U	Uridine monophosphate UMP
	Thymine T	Thymidine T	Thymidine monophosphate TMP

Polynucleotides

- Are polymers of nucleotides which are formed by the **Condensation of Nucleotides**
- The condensation reaction occurs between the **alcohol of a 5'- phosphate** of one nucleotide and the **3'-hydroxyl** of the second, with the elimination of **H_2O**
- The linkage between 2 nucleotides is known as **Phosphodiester Bond**
- Each nucleotide that has been incorporated into the polynucleotide is known as a **Nucleotide Residue**
- The phosphates of polynucleotides are **Acidic**, so at physiological pH,

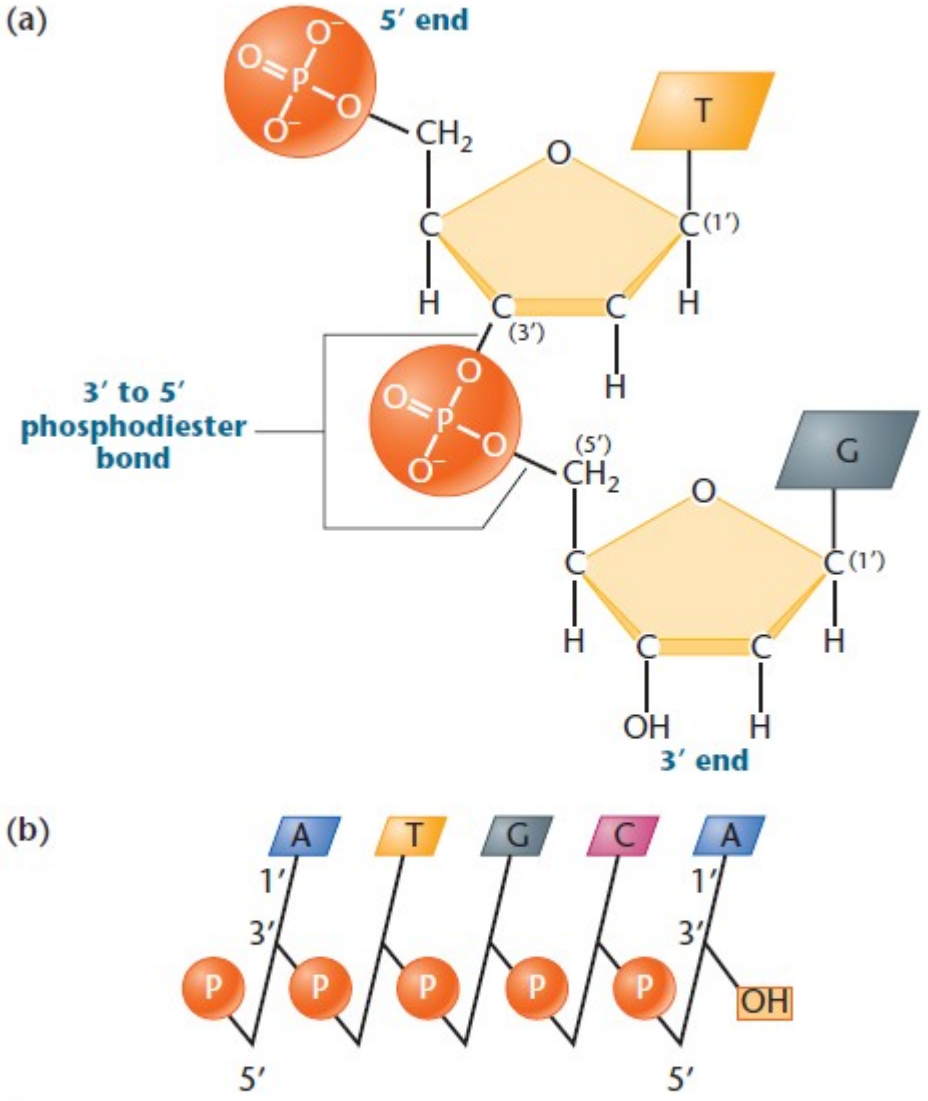


FIGURE 10–10 (a) Linkage of two nucleotides by the formation of a C-3′-C-5′ (3′-5′) phosphodiester bond, producing a dinucleotide. (b) Shorthand notation for a polynucleotide chain.

- The formation of phosphodiester bonds in DNA and RNA exhibits directionally
- The primary structure of DNA and RNA (linear arrangements of the nucleotides)
- The common representation of the primary structure of DNA /RNA molecules is to write the nucleotide sequences from left to right synonymous with the 5'→3' direction
- The **interchain/ intrachain base-pairing of complementary nucleotides** gives the secondary structure of a nucleic acid
- The 3D arrangement of nucleic acid- i.e. the arrangement of the molecule in space refers to

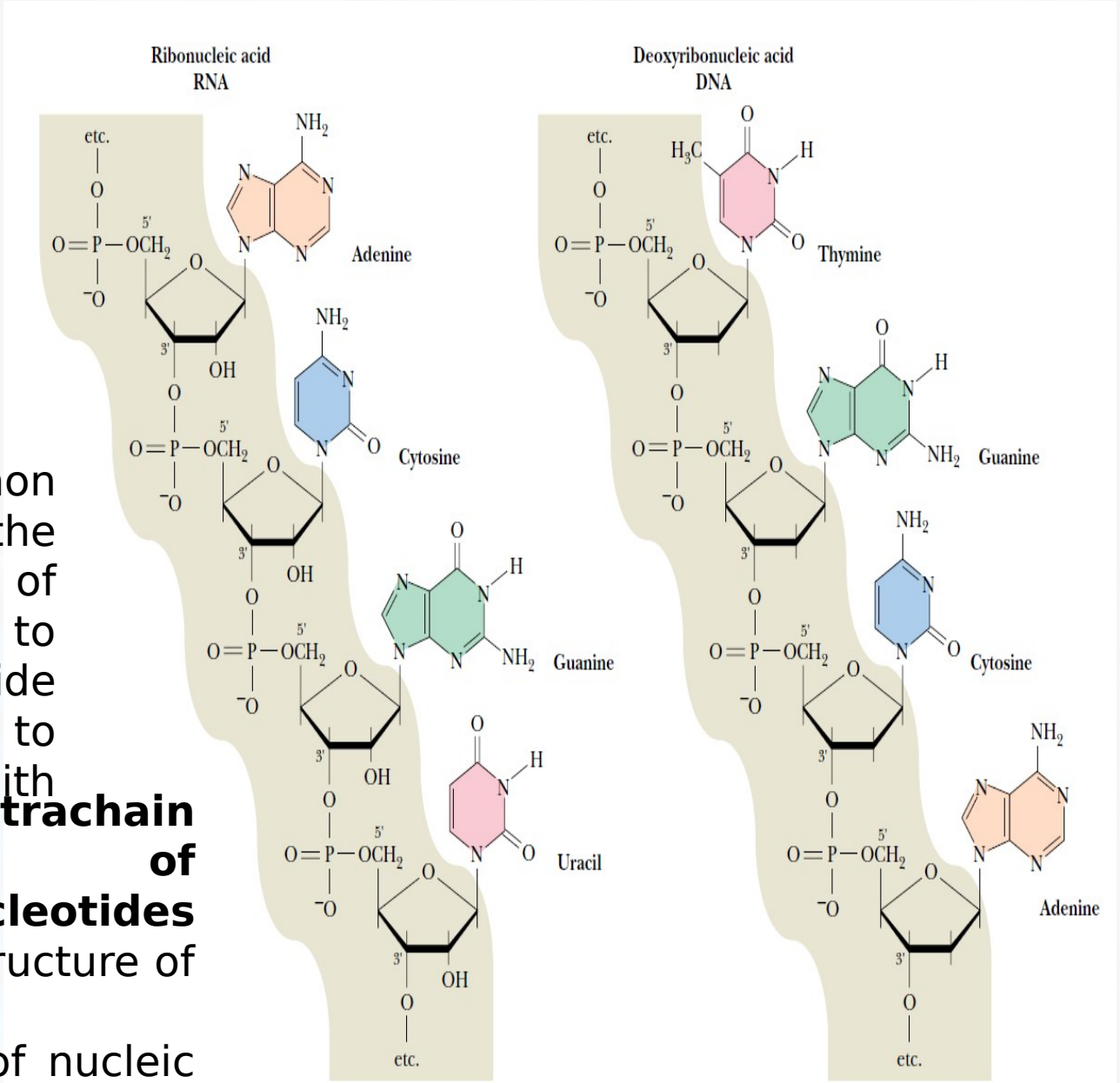
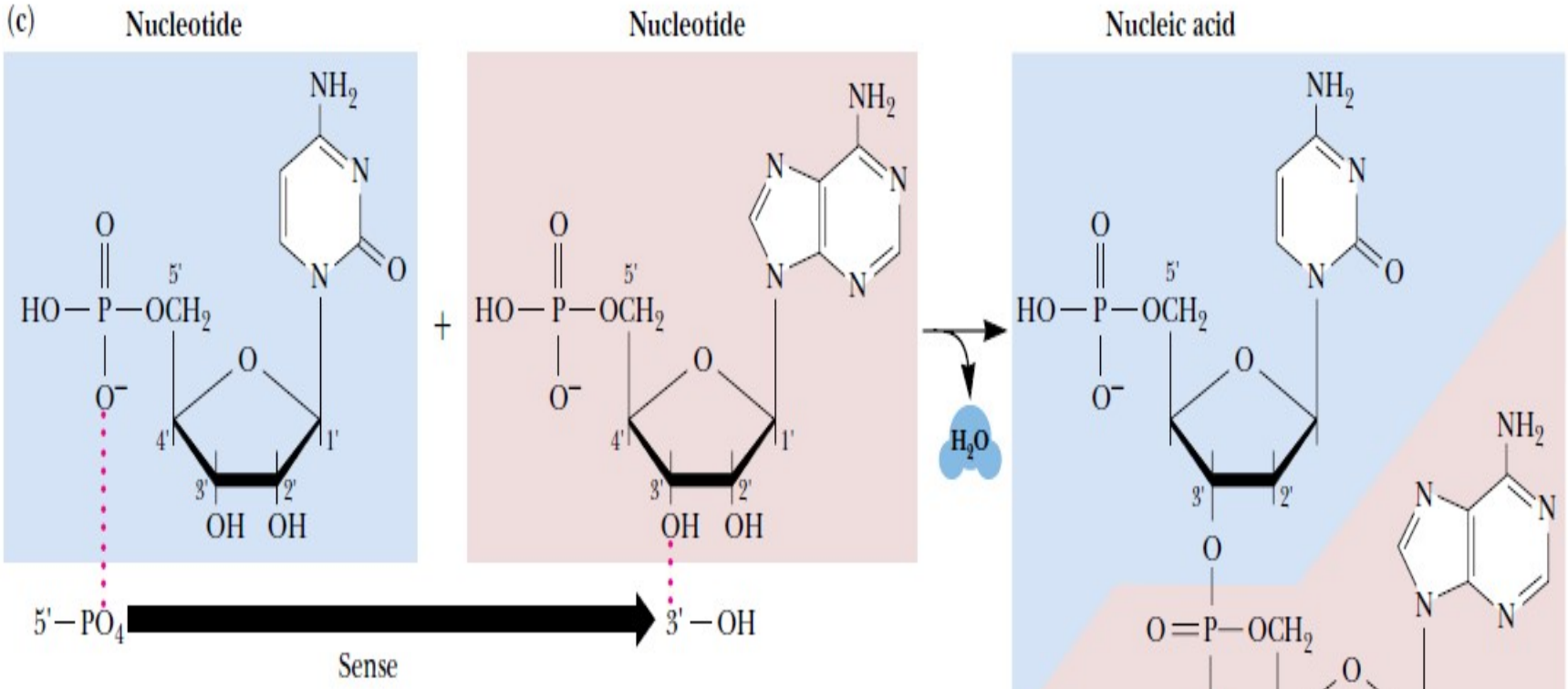
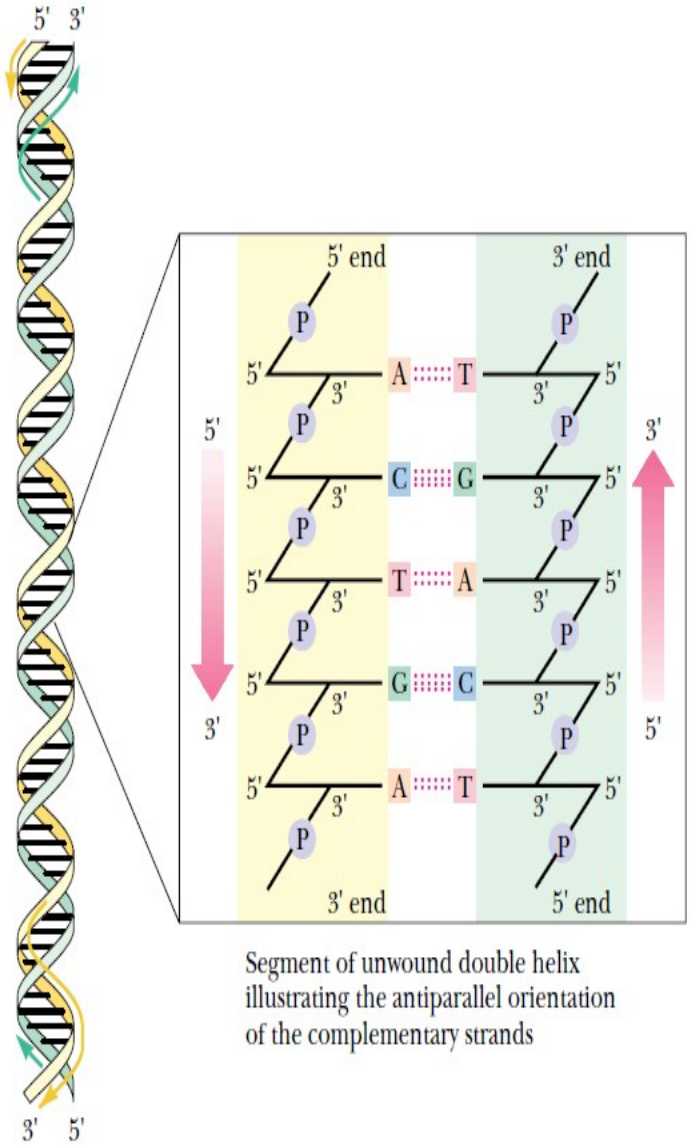


FIGURE 11.17 • 3′-5′ phosphodiester bridges link nucleotides together to form polynucleotide chains.

proceeds in the **5'→3' direction**


(c)
Nucleotide
Nucleotide
Nucleic acid
NH_2
N
O
HO—P—OCH_2
5'
O^-
4'
1'
3'
2'
OH OH
+
H_2O
5'—PO_4
3'—OH
Sense

Chargaff's rules



Segment of unwound double helix illustrating the antiparallel orientation of the complementary strands

- **Erwin Chargaff** proposed 2 rules which is termed as **Chargaff's rules**
- These rules played an important role in the discovery of the double helix structure of DNA
- **RULE 1**- In any double stranded DNA the number of **Guanine** units equals the number of **Cytosine** units and the number of **Adenine** units equals the number of **Thymine** units
- **RULE 2** – The composition of DNA varies from one species to another
- Hence, In dsDNA :
  - The molar ration of **A** and **T** equals to **1**
  - The molar ration of **G** to **C** equals to **1**
  - The sum of **Purines (A** and **G)** equals that of the **Pyrimidines (C** and **T)**
  - The percentage of **C+G** does not

FIGURE 11.19 • The antiparallel nature of the DNA double helix.

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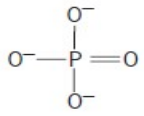
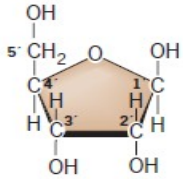
Nucleic acids are composed of repeating subunits called nucleotides. Each nucleotide is composed of three units.

(1) A phosphate group:

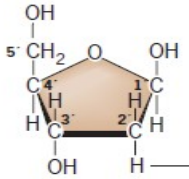


(2) A five-carbon sugar or pentose:

(a) In RNA: Ribose

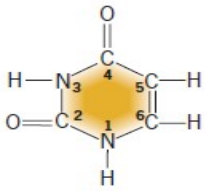


(b) In DNA: 2-Deoxyribose



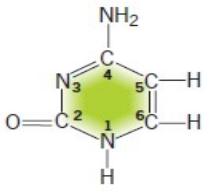
(3) A cyclic, nitrogen-containing base:

(a) In RNA only (with rare exceptions):



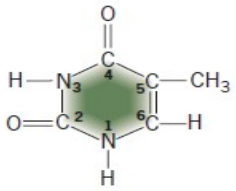
Uracil

(b) In both RNA and DNA:



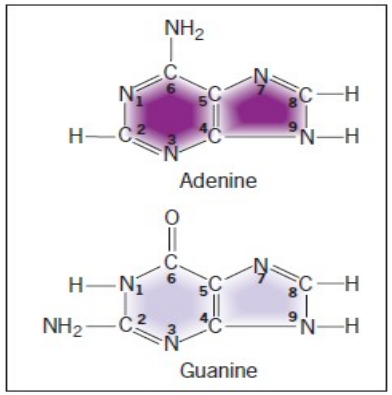
Cytosine

(c) In DNA only (with rare exceptions):



Thymine

Pyrimidines



Adenine

Guanine

Purines

■ **FIGURE 9.5** Structural components of nucleic acids. The standard numbering systems for the carbons in pentoses and the carbons and nitrogens in the ring structures of the bases are shown in (2) and (3), respectively. Single-ring bases are called pyrimidines, and double-ring-bases are purines.

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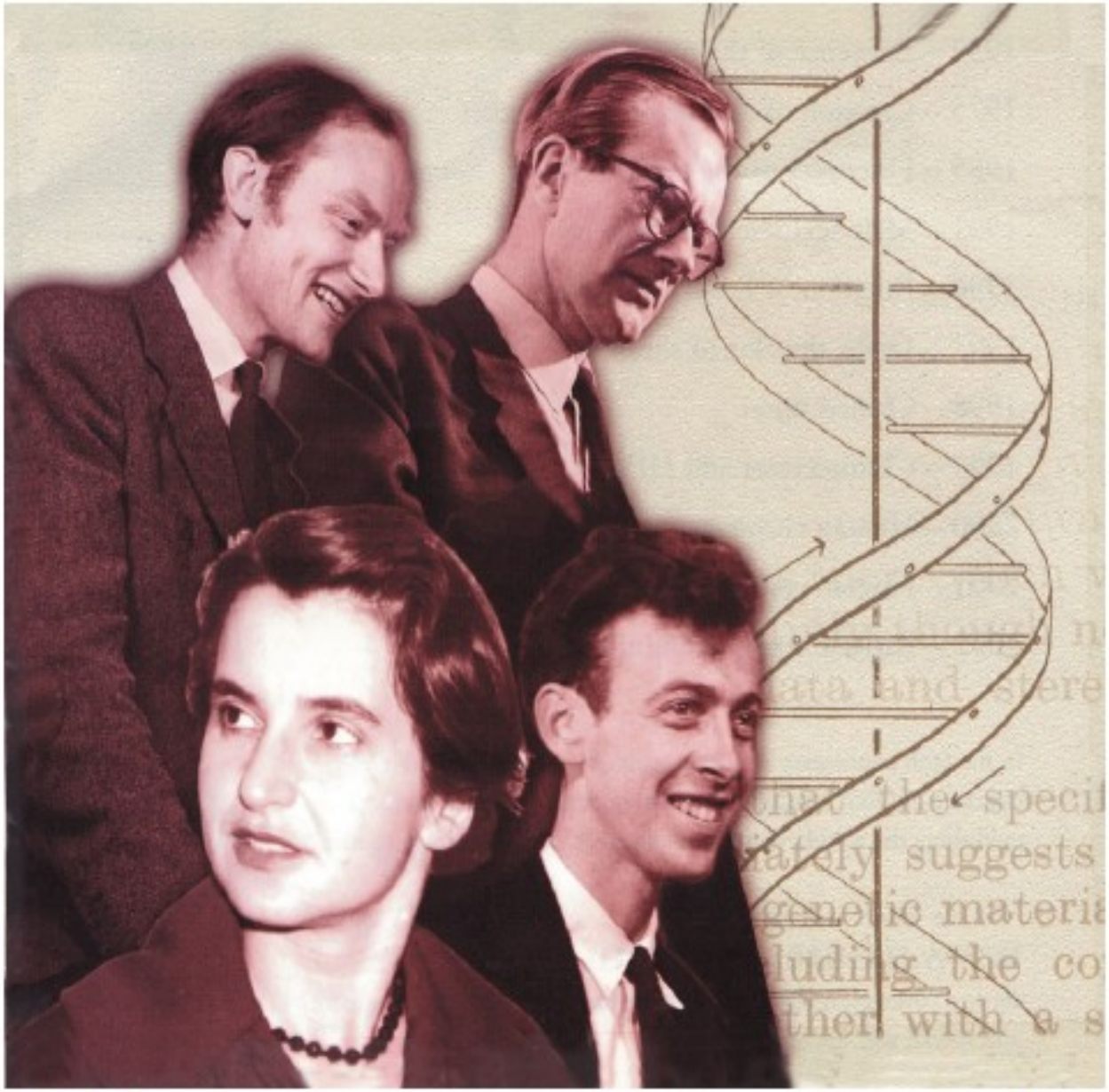
Table 11.3

Molar Ratios Leading to the Formulation of Chargaff's Rules

Source	Adenine to Guanine	Thymine to Cytosine	Adenine to Thymine	Guanine to Cytosine	Purines to Pyrimidines
Ox	1.29	1.43	1.04	1.00	1.1
Human	1.56	1.75	1.00	1.00	1.0
Hen	1.45	1.29	1.06	0.91	0.99
Salmon	1.43	1.43	1.02	1.02	1.02
Wheat	1.22	1.18	1.00	0.97	0.99
Yeast	1.67	1.92	1.03	1.20	1.0
Hemophilus influenzae	1.74	1.54	1.07	0.91	1.0
E. coli K-12	1.05	0.95	1.09	0.99	1.0
Avian tubercle bacillus	0.4	0.4	1.09	1.08	1.1
Serratia marcescens	0.7	0.7	0.95	0.86	0.9
Bacillus schatz	0.7	0.6	1.12	0.89	1.0

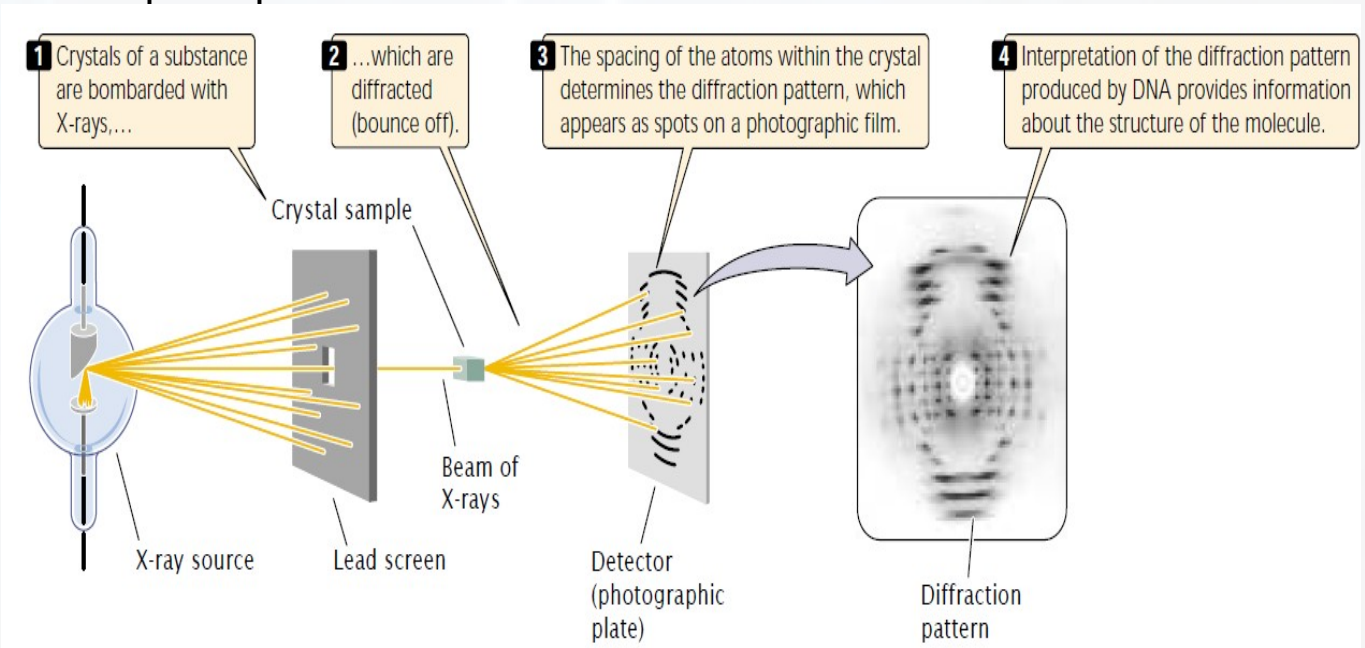
Source: After Chargaff, E., 1951. *Federation Proceedings* **10**:654–659.



■ **FIGURE 9.8** The four major players—Francis Crick, Maurice Wilkins, James Watson, and Rosalind Franklin (clockwise from top left)—in the discovery of the double-helix structure of DNA.

Structure of DNA

- **Watson** and **Crick** first described the structure of the **DNA double helix** in **1953** using **X-ray diffraction data** of DNA fibers obtained by **R Franklin** and **M Wilkins**
- Watson, Crick, Wilkins were awarded the 1962 Nobel prize for medicine for discovering the molecular structure of DNA
- The Watson-Crick double helix model describes the features of **B -form of DNA**
- However, there are many other forms or conformations of DNA (such as **A-, C-, Z-forms**) which are very distinct from the **B form**
- The form that DNA would adopt depends on several factors:
  - **Hydration level**
  - **DNA sequence**
  - **Chemical modifications of the bases**
  - **The type and concentration of metal ions in solution**


1 Crystals of a substance are bombarded with X-rays,...
2 ...which are diffracted (bounce off).
3 The spacing of the atoms within the crystal determines the diffraction pattern, which appears as spots on a photographic film.
4 Interpretation of the diffraction pattern produced by DNA provides information about the structure of the molecule.
Crystal sample
Beam of X-rays
X-ray source
Lead screen
Detector (photographic plate)
Diffraction pattern

10.6 **X-ray diffraction provides information about the structures of molecules.** (Photo from M. H. F. Wilkins, Department of Biophysics, King's College, University of London.)

Stability of the DNA helix

The helical structure of dsDNA is stabilized by non covalent interactions

These interactions include,

- **Stacking interactions(major) between adjacent bases**
- **Hydrogen bonding (minor) between complementary strands**

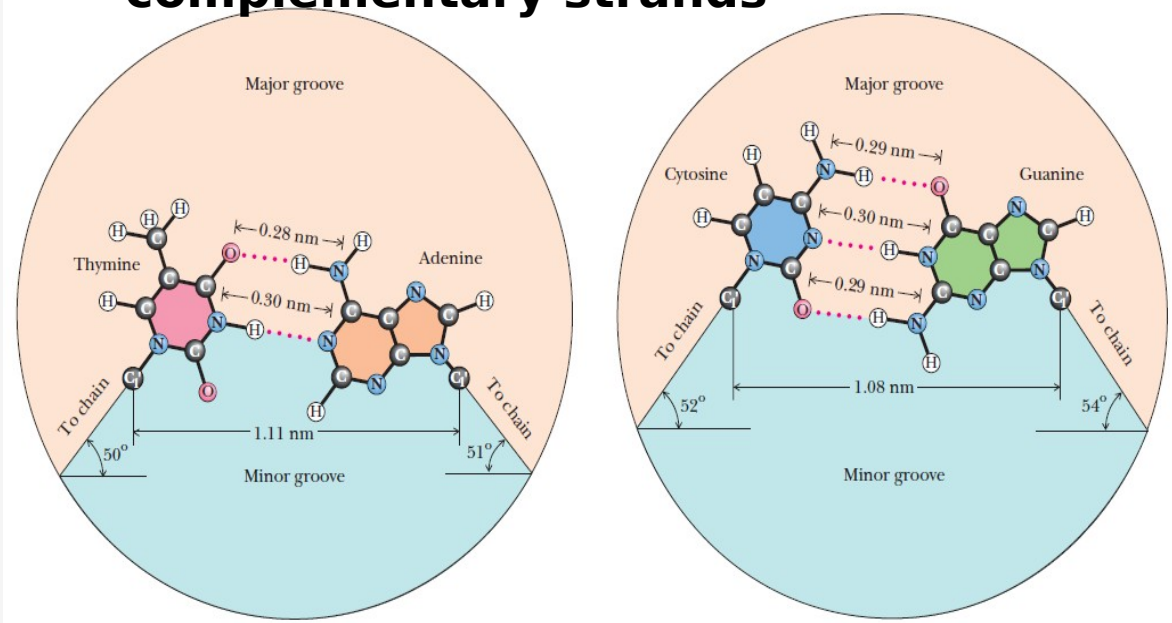


FIGURE 12.10 • Watson–Crick A:T and G:C base pairs. All H bonds in both base pairs are straight, with each H atom pointing directly at its acceptor N or O atom. Linear H bonds are the strongest. The mandatory binding of larger purines with smaller pyrimidines leads to base pairs that have virtually identical dimensions, allowing the two sugar–phosphate backbones to adopt identical helical conformations.

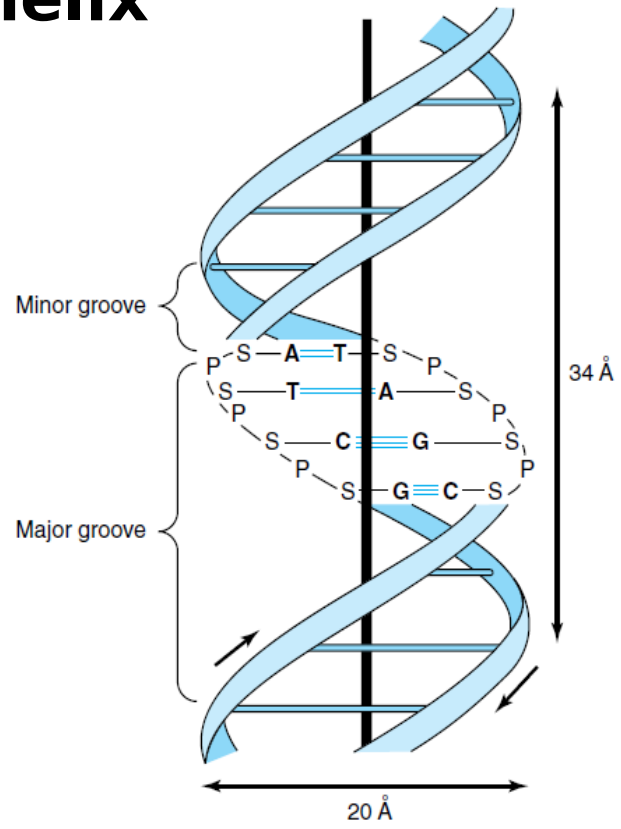
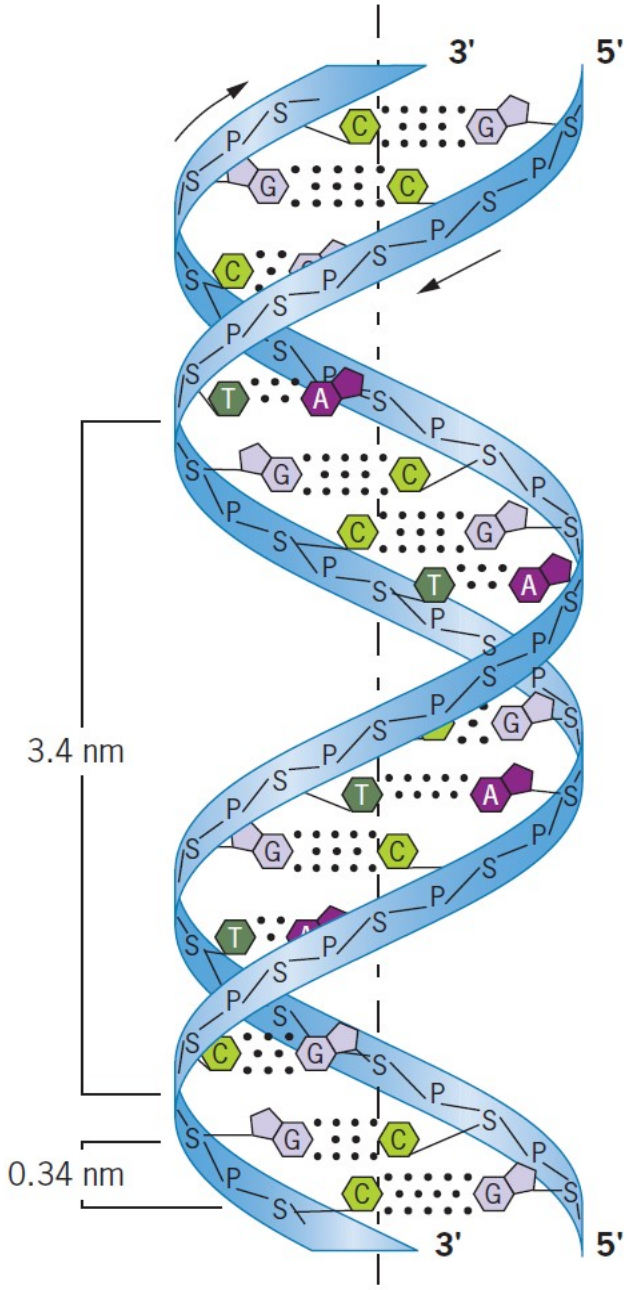


Figure 35–2. A diagrammatic representation of the Watson and Crick model of the double-helical structure of the B form of DNA. The horizontal arrow indicates the width of the double helix (20 Å), and the vertical arrow indicates the distance spanned by one complete turn of the double helix (34 Å). One turn of B-DNA includes ten base pairs (bp), so the rise is 3.4 Å per bp. The central axis of the double helix is indicated by the vertical rod. The short arrows designate the polarity of the antiparallel strands. The major and minor grooves are depicted. (A, adenine; C, cytosine; G, guanine; T, thymine; P, phosphate; S, sugar [deoxyribose].)



■ **FIGURE 9.10** Diagram of the double-helix structure of DNA.

- The core of the helix consists of the base pairs which stack together through stacking interactions = **Hydrophobic Interactions** and **Van Der Waals Interactions**
- Base stacking also helps to minimize contact of the bases with water
- Internal and external hydrogen bonds also stabilize the double helix
- 2 strands of DNA are held together by **Hydrogen Bonds** that form between the complementary purines and pyrimidines, 2 hydrogen bonds in an **A-T** and **C-G** base pairs

Base stacking energies depend on the sequence of the DNA. Some combinations of base pairs form more stable interactions than others

- Ex. A (GC). (GC) dinucleotide stack has a stacking energy of -14.59 kcal/mol/stacked pair, whereas (TA).(TA) stack has an energy of -3.82kcal/mol/stacked pair
- Once the DNA double helix is formed, it is remarkably stable. The individual interactions stabilizing the helix are weak, but the sum of all interactions makes a

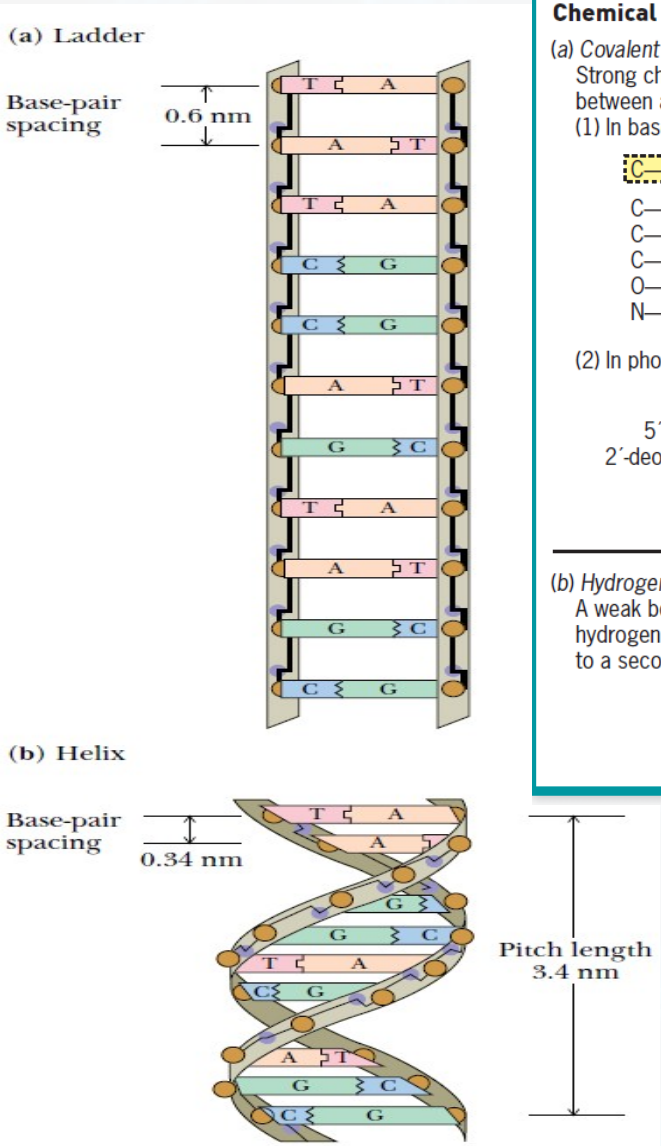


FIGURE 12.9 • (a) Double-stranded DNA as an imaginary ladderlike structure. (b) A simple right-handed twist converts the ladder to a helix.

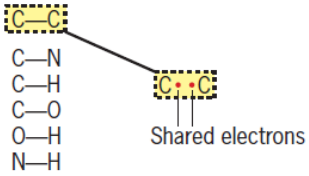
TABLE 9.2

Chemical Bonds Important in DNA Structure

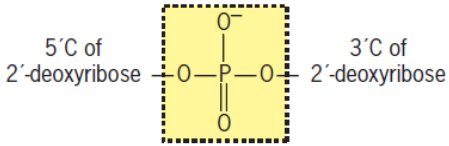
(a) Covalent bonds

Strong chemical bonds formed by sharing of electrons between atoms.

(1) In bases and sugars

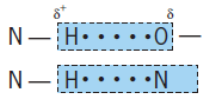


(2) In phosphodiester linkages



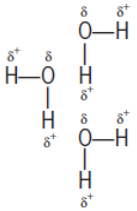
(b) Hydrogen bonds

A weak bond between an electronegative atom and a hydrogen atom (electropositive) that is covalently linked to a second electronegative atom.



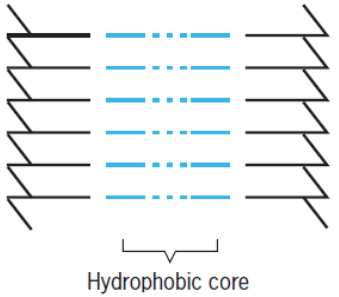
(c) Hydrophobic "bonds"

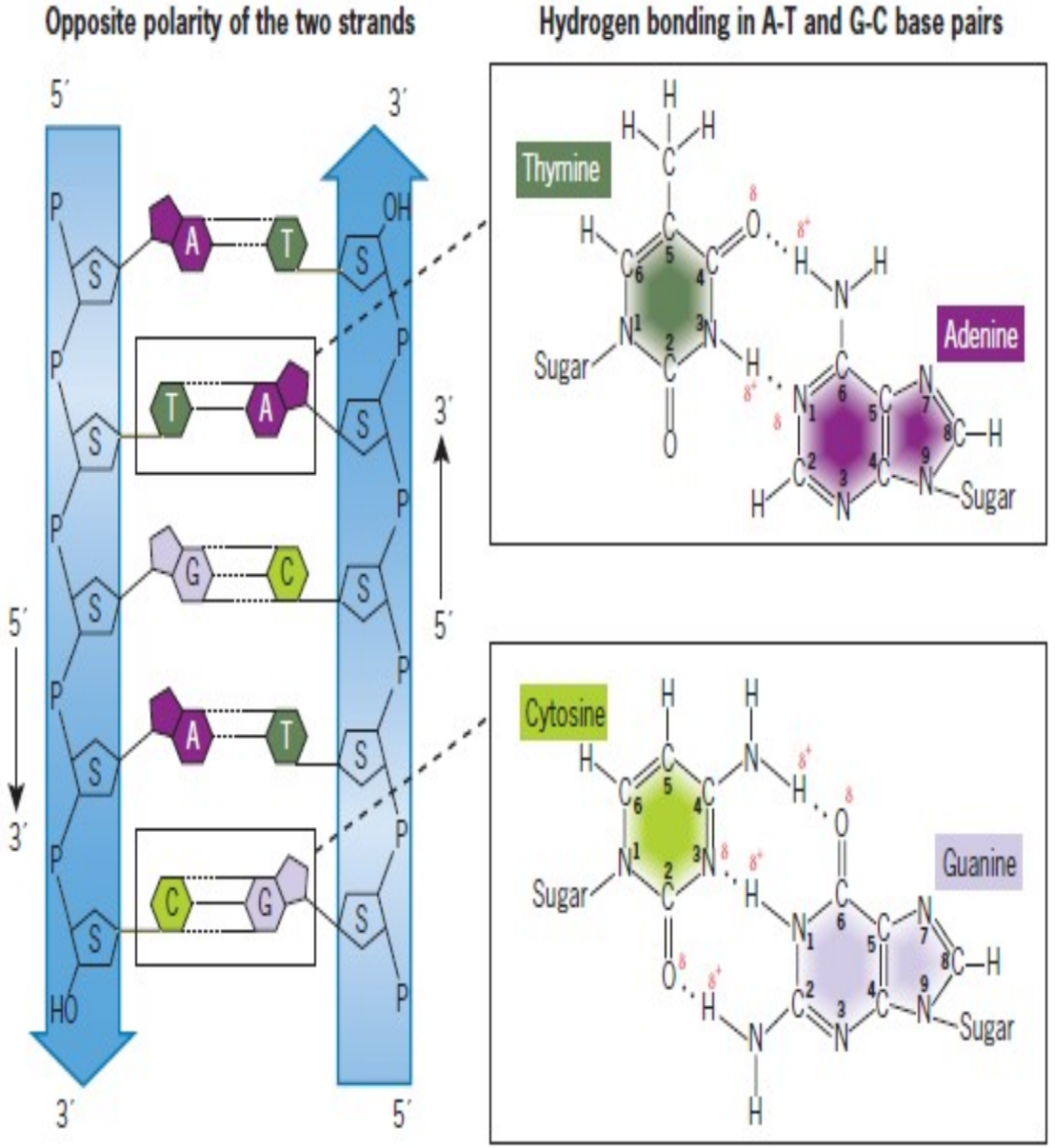
The association of nonpolar groups with each other when present in aqueous solutions because of their insolubility in water.



Water molecules are very polar (δ^- O and δ^+ H's). Compounds that are similarly polar are very soluble in water ("hydrophilic"). Compounds that are nonpolar (no charged groups) are very insoluble in water ("hydrophobic").

The stacked base pairs provide a hydrophobic core.





■ **FIGURE 9.11** Diagram of a DNA double helix, illustrating the opposite chemical polarity (see Figure 9.7) of the two strands and the hydrogen bonding between thymine (T) and adenine (A) and between cytosine (C) and guanine (G). The base-pairing in DNA, T with A and C with G, is governed by the hydrogen-bonding potential of the bases. S = the sugar 2-deoxyribose; P = a phosphate group.

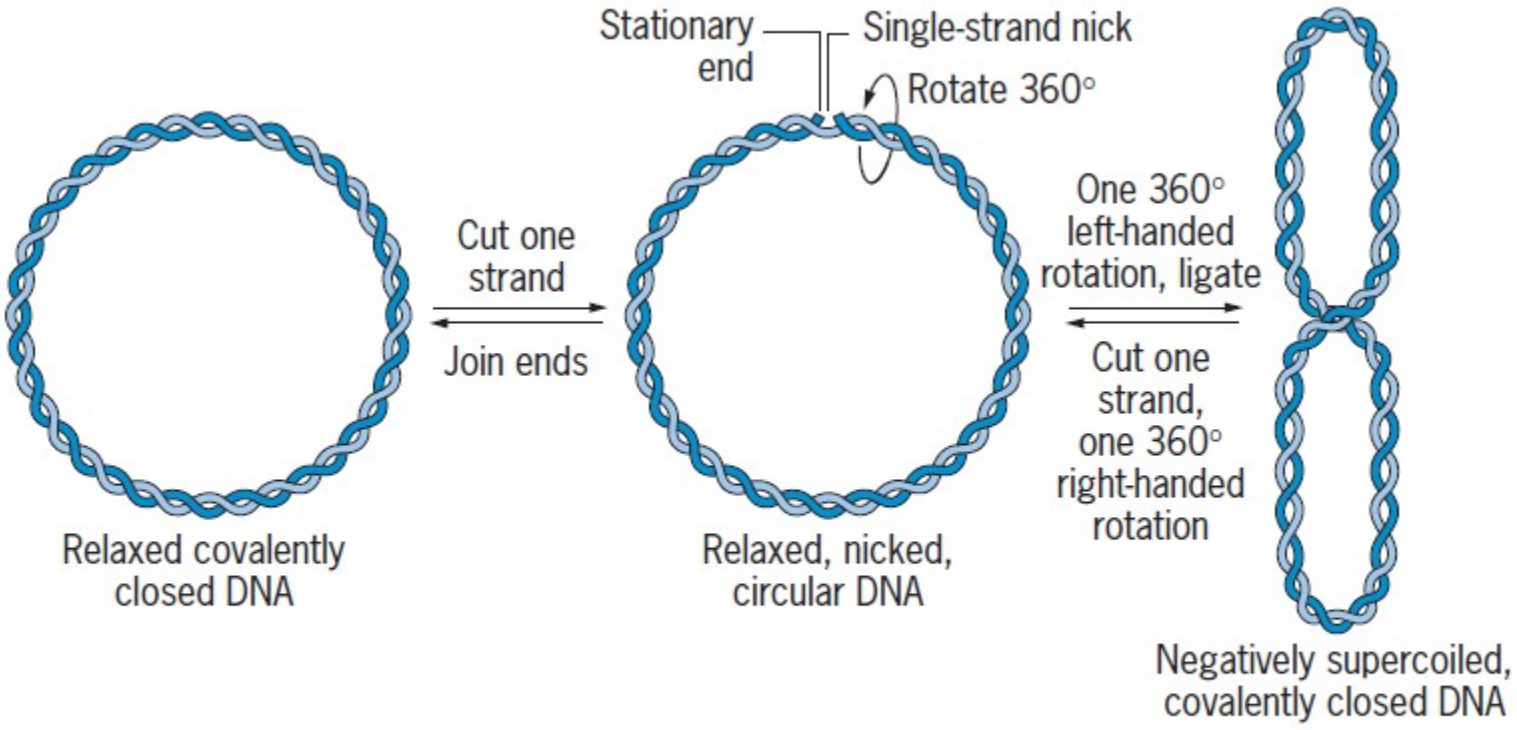
ferent forms of DNA and its key features

Geometry attributes	A-form (transposons/ jumping genes)	B-form (Common)	Z-form (Zig zag form)
Helix sense	Right handed	Right handed	Left handed
Repeating unit	1bp	1bp	2bp
Rotation/BP (twist angle)	33.6°	34.3°	60°/2
Mean BP/turn	10.7	10.4	12
Base pair tilt	20°	-6°	7°
Rise/BP along axis	2.3Å	3.32Å	3.8Å
Diameter	23Å	20Å	18Å
Major groove	Narrow and deep	Wide and deep	flat
Minor groove	Wide and shallow	Narrow and deep	Narrow and deep

Supercoiled forms of DNA:

- **Supercoiling-** coiling of a coil
- A coiling of DNA double helix upon itself
- Can occur in **covalently closed, circular DNAs** and also in **linear DNAs** that are topologically constrained by being complexed with protein
- In linear DNAs, if the ends of the molecule are fixed then any rotationally freedom that the two backbones might have about one another is removed and as a result any local twisting or untwisting will cause strain on the structure
- DNA supercoiling is generally a **manifestation of structural strain**
- If there is **no net bending** of the DNA axis upon itself, the DNA is said to be in **Relaxed State**
- The increase or decrease in the number of base pairs per helical turn creates a state of torsional stress in the helix
- This stress is relieved by supercoiling of the helix
- The supercoiling can be **Positive or Negative**

- **Positively supercoiled DNA-** is **overwound** (has **more helical turns** than what the molecule would accommodate in relaxed state), with **less base pairs per helical turn** than relaxed DNA (i.e. <10.4 bp per helical turn)
- **Negatively supercoiled DNA-** is **underwound** (has **fewer helical turns** that what molecule would accommodate in relaxed state), with **more base pairs per helical turn** than relaxed DNA (i.e.. >10.4 bp per helical turn)
- Most DNA in organisms is found in **Negatively Supercoiled State** with a deficiency of helical turns
- The superhelix is left **handed** in **positively supercoiled B-DNA**
- The negative supercoiling may be **Solenoidal** and **Plectonemic** (or interwound)
- The **Plectonemic superhelix** is **right handed** and is characteristics of



■ **FIGURE 9.14** A visual definition of negatively supercoiled DNA. Although the structure of DNA supercoils is most clearly illustrated by the mechanism shown here, DNA supercoils are produced by a different mechanism *in vivo* (see Chapter 10).

DNA denaturation

- Is a process in which ds DNA separates into 2 single strands due to disruption of hydrogen bonds stacking interactions i.e. it is a process of the separation of DNA strands
- Several factors like extreme pH, temperature, ionic strength causes DNA denaturation
- This process is accompanied by a change in DNA's physical properties
- Denaturation increases the relative absorbance of the DNA solution at 260nm
- This increases in the absorbance and called **Hyper Chromic Shift**

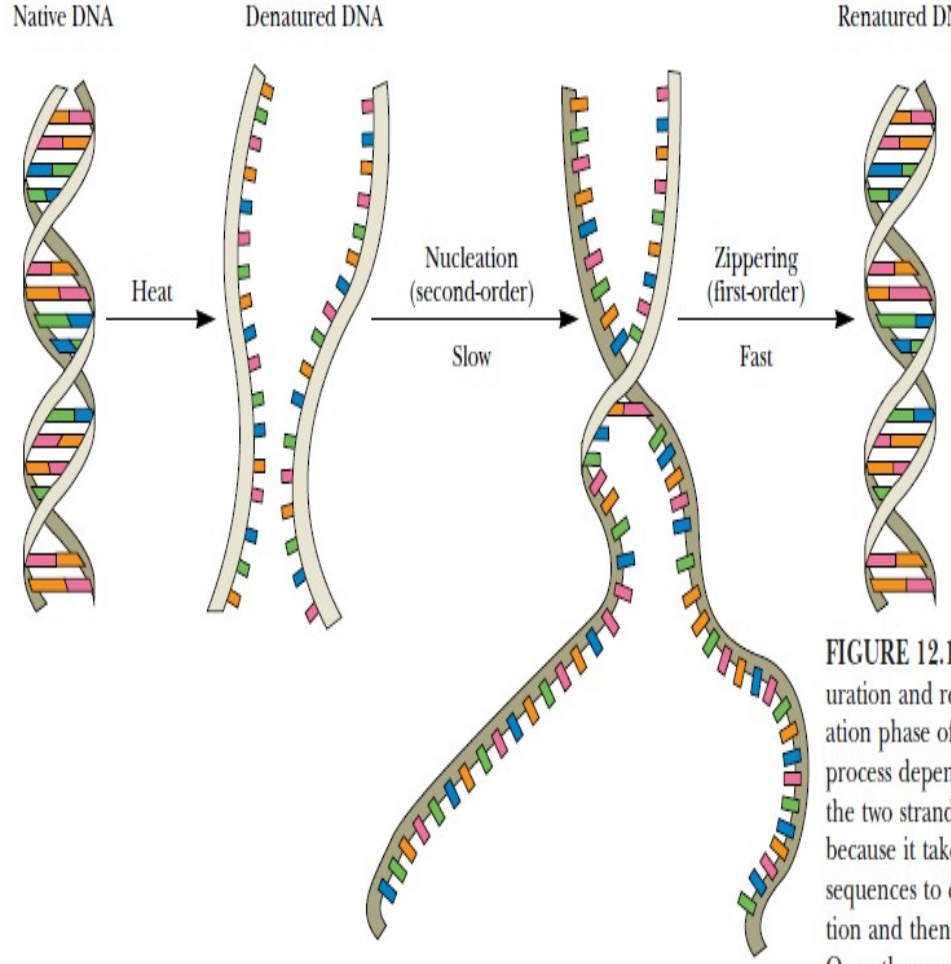


FIGURE 12.19 • Steps in the thermal denaturation and renaturation of DNA. The nucleation phase of the reaction is a second-order process depending on sequence alignment of the two strands. This process takes place slowly because it takes time for complementary sequences to encounter one another in solution and then align themselves in register. Once the sequences are aligned, the strands zipper up quickly.

Stacked bases in dsDNA absorbs less at 260nm than unstacked bases. The increased absorbance is due to the fact that the aromatic bases in DNA interact via their π-electron clouds when stacked together in the double helix. Because the absorbance of bases at 260nm is a consequence of π-electron transitions, and because the potential for these transitions is diminished when the bases stack, the bases in duplex DNA absorb less at 260 nm than expected. The rise in absorbance coincides with strand

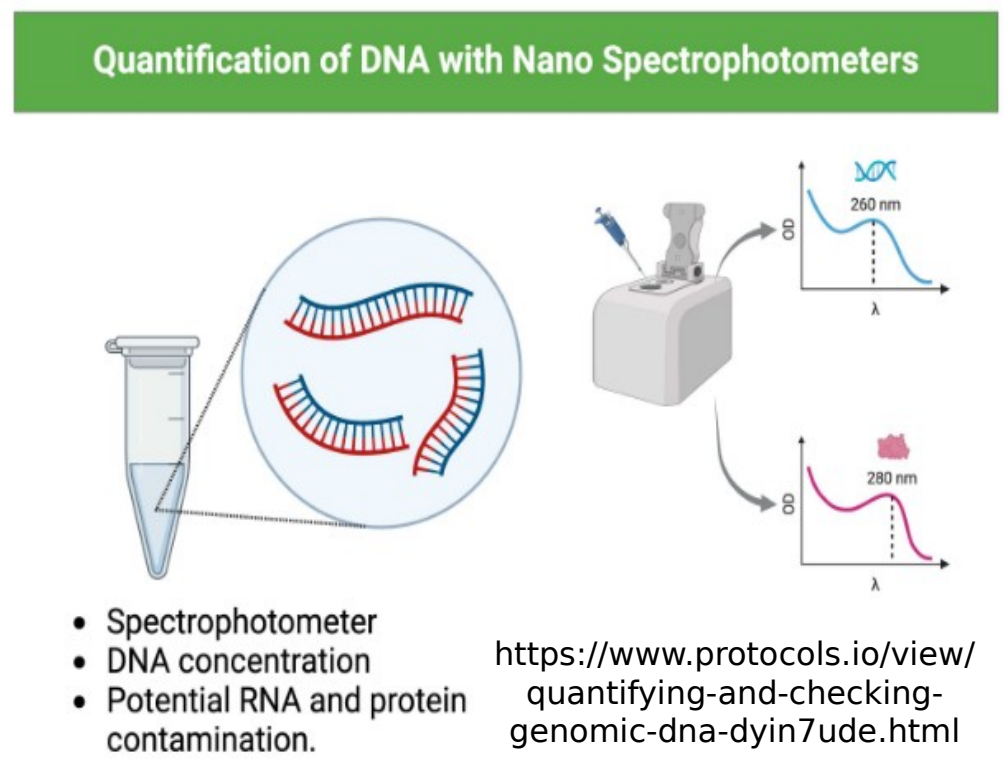
Factors affecting T_m

- The temperature at which half of the dsDNA is denatured is called **T_m (melting temperature).** The T_m value(or the thermal stability of DNA double helix) depends on several factors, including ionic strength, GC content and pH
- **GC content-**
  (↑) GC content = **(↑)** T_m
  (↓) GC content = **(↓)** T_m
- **Ionic strength-**(DNA is a polyanionic molecule. Each phosphate group in a DNA strand carries a negative charge)
  - The negative charges on both strands of dsDNA repel each other.

  (↓) ionic strength= **(↓)** melting temperature
  (↑) ionic strength= **(↑)** melting temperature
- **pH-**
  - At pH values **>10** = extensive deprotonation of the bases occurs = destroying their hydrogen bonding potential = denaturing the DNA duplex
  - At pH values **<3** = extensive protonation of the bases occurs = disrupts base pairing

Quantification of nucleic acids

- Ultraviolet spectroscopy is used for quantification of nucleic acids
- Nucleic acids= shows a strong absorbance in region of 240-275nm
- It originates from the π→π * transitions of pyrimidine and purine ring systems of the nucleobases
- Bases can be protonated- therefore spectra of DNA and RNA are sensitive to pH
- At neutral pH:
  - Absorption maxima range,
  - 253nm - for **Guanine**
  - 271nm - for **Cytidine**



- Polymeric DNA and RNA show a broad and strong absorbance near 260 nm
- At this wavelength absorption is proportional to nucleic acid concentration
- The relationship is so well characterized that UV absorption is used to accurately determine the concentration of nucleic acids in solution
- The relationship between DNA concentration and absorption is linear
- The absorbance at 260nm is used to determine the concentration of nucleic acids
- Amounts of nucleic acid are often given as A_{260} units
- For double-stranded DNA = 1 A_{260} unit is equivalent to 50 µg DNA (a solution of DNA with a concentration of 50 µg/ml will have an absorbance at 260nm equal to 1.0) for single-stranded DNA, it is equivalent to 33 µ g DNA; for single-stranded RNA, it is equivalent to 40 µg RNA

DNA- a genetic material

- It contains the genetic instructions specifying the biological development of all cellular forms of life, and many viruses
- DNA acts as genetic material and used to store the genetic information of an organic life form
- DNA encodes the sequence of the amino acid residues in proteins using the genetic code, a triplet code of nucleotides
- For all currently known living organisms, the genetic material is almost exclusively DNA
- Some viruses use RNA as their genetic material
- The first genetic material is generally believed to have been RNA, initially manifested by self-replicating RNA molecules floating on bodies of water
- This hypothetical period in the evolution of cellular life is known as the **RNA world**
- This hypothesis is based on the RNA's ability to act both as genetic material and as a catalyst, known as **Ribozyme** or a ribosome.
- However once proteins, which can form enzymes, came into existence, a more stable molecule DNA became the dominant genetic material, a situation continued today
- Not only does DNA's double-stranded nature allows for correction of mutations but RNA is inherently unstable
- Modern cells use RNA mainly for the building of proteins from DNA

Griffith's Experiment

- In 1928, Griffith performed transformation experiments with two different strains of the bacterium ***Diplococcus pneumoniae*** (the organism is now named ***Streptococcus pneumoniae***) **– virulent strains**, which cause **Pneumonia** in certain vertebrates (such as **Mice**, **Human**) and **avirulent strains**, which do not cause **Pneumonia**.
- The difference in virulence is related to the polysaccharides capsule of the bacterium:
  - **Presence of capsule= Virulent strains**
  - **Absence of capsule= Avirulent strains**
- The **Non-capsulated Bacteria** are readily engulfed and destroyed by phagocytic cells
- The **Capsulated bacteria** are not readily engulfed and Hence, they are able to multiply and cause pneumonia
- The **Encapsulated bacteria** form smooth colonies **(S)** when grown on an agar culture plate
- **Non-encapsulated bacteria** produce rough colonies **(R)** when grown on an agar culture plate
- Each strain of ***Diplococcus*** may be of different serotypes- **IS**, **IIS**, **IIIS** or **IR**, **IIR** and **IIIR**
- 2 serotypes of ***Diplococcus*** were used in experiment (**IIR** and **IIIS**)
- Serotypes are identified by immunological techniques

SEROTYPE	MORPHOLOGY	CAPSULE	VIRULENCE
IIR	Rough	Absent	Avirulent
IIIS	Smooth	Present	Virulent

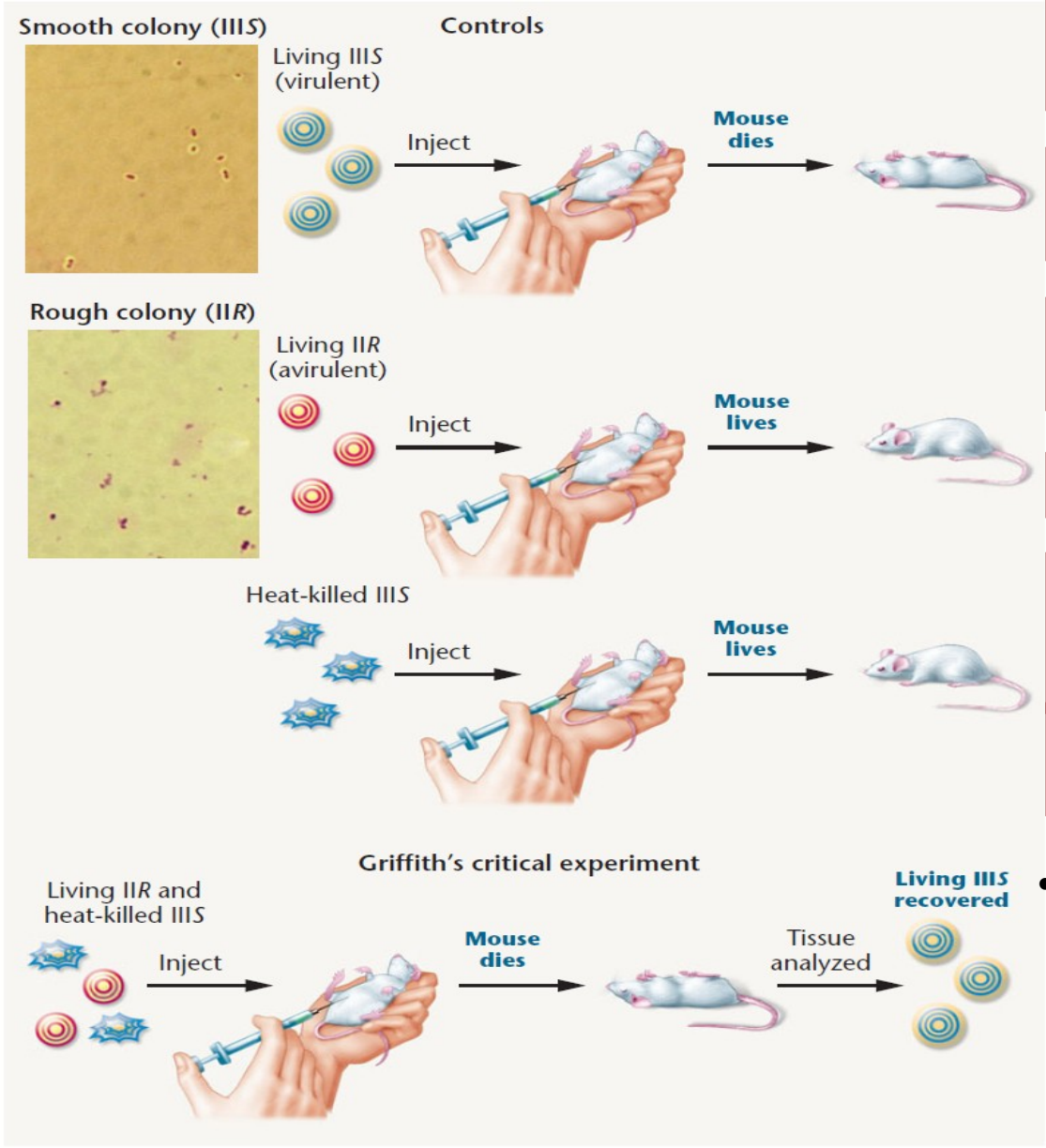


FIGURE 10–2 Griffith's transformation experiment. The photographs show bacterial colonies containing cells with capsules (type IIIS) and without capsules (type IIR).

Griffith injected the different strains of bacteria into mice

↓

The IIIS strain killed the mice
The IIR strain did not

↓

The heat killed IIIS strain was injected into a mouse

↓

It did not cause pneumonia

↓

He combined heat-killed IIIS with live IIR and injected the mixture into a mouse

↓

The mouse developed pneumonia and died

- Griffith concluded that the heat-killed **IIIS** bacteria were responsible for converting live avirulent IIR cells into virulent **IIIS** ones and called the phenomenon **transformation**
- He called the genetic information which could be

Avery- Macleod- McCarty Experiment

- In 1944, **Oswald Avery, Colin MacLeod** and **Maclyn McCarty** revisited Griffith's experiment
- They conducted that the transforming material was pure DNA not protein or RNA
- These investigators found that DNA extracted from a virulent strain of the bacterium ***Streptococcus pneumoniae***, also known as ***pneumococcus***, genetically transformed an **avirulent strain** of this organism into a **virulent form**

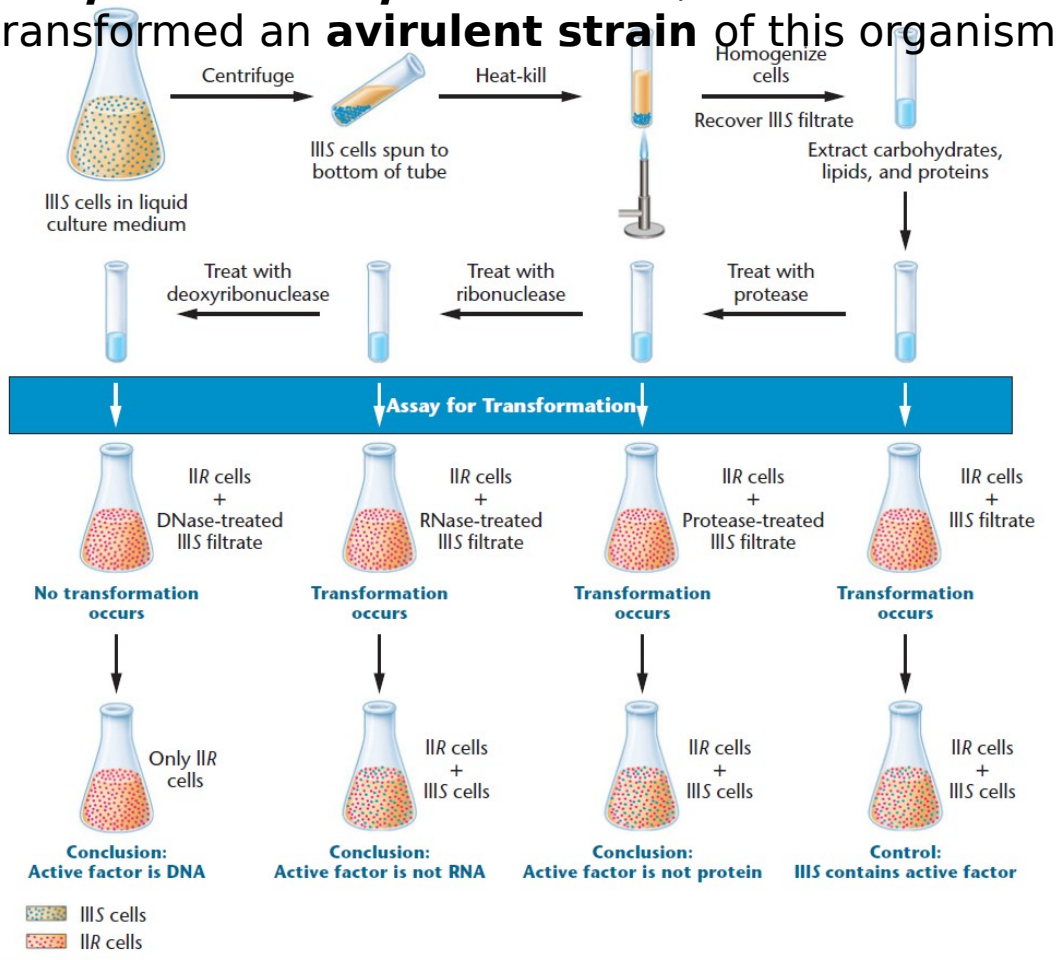


FIGURE 10–3 Summary of Avery, MacLeod, and McCarty's experiment demonstrating that DNA is the transforming principle.

Type IIR cells
No Transformation

Type IIR cells+ type IIIS DNA **Extract**
Transformation

Type IIR cells+ type IIIS DNA extract+ DNase
No Transformation

Type IIR cells+ type IIIS DNA extract+ RNase
Transformation

Type IIR cells+ type IIIS DNA extract+ protease
Transformation

Hershey-chase experiment

- Conducted in 1952 by **Alfred Hershey** and **Martha Chase**
- This experiment identified DNA to be the genetic material of **phages**
- A **phage** is a virus that infects bacteria
- It consists of a **protein coat** that encloses the **dsDNA**
- Since phages consist **only of nucleic acid** surrounded by **protein**, they lend themselves nicely to the determination of whether the protein or the nucleic acid is the genetic material
- Hershey and Chase designed an experiment using **radioactive isotopes of sulfur** and **phosphorus** to keep separate track of the viral proteins and nucleic acids during infection process
- They used the **T_2 bacteriophage** and the bacterium ***Escherichia***

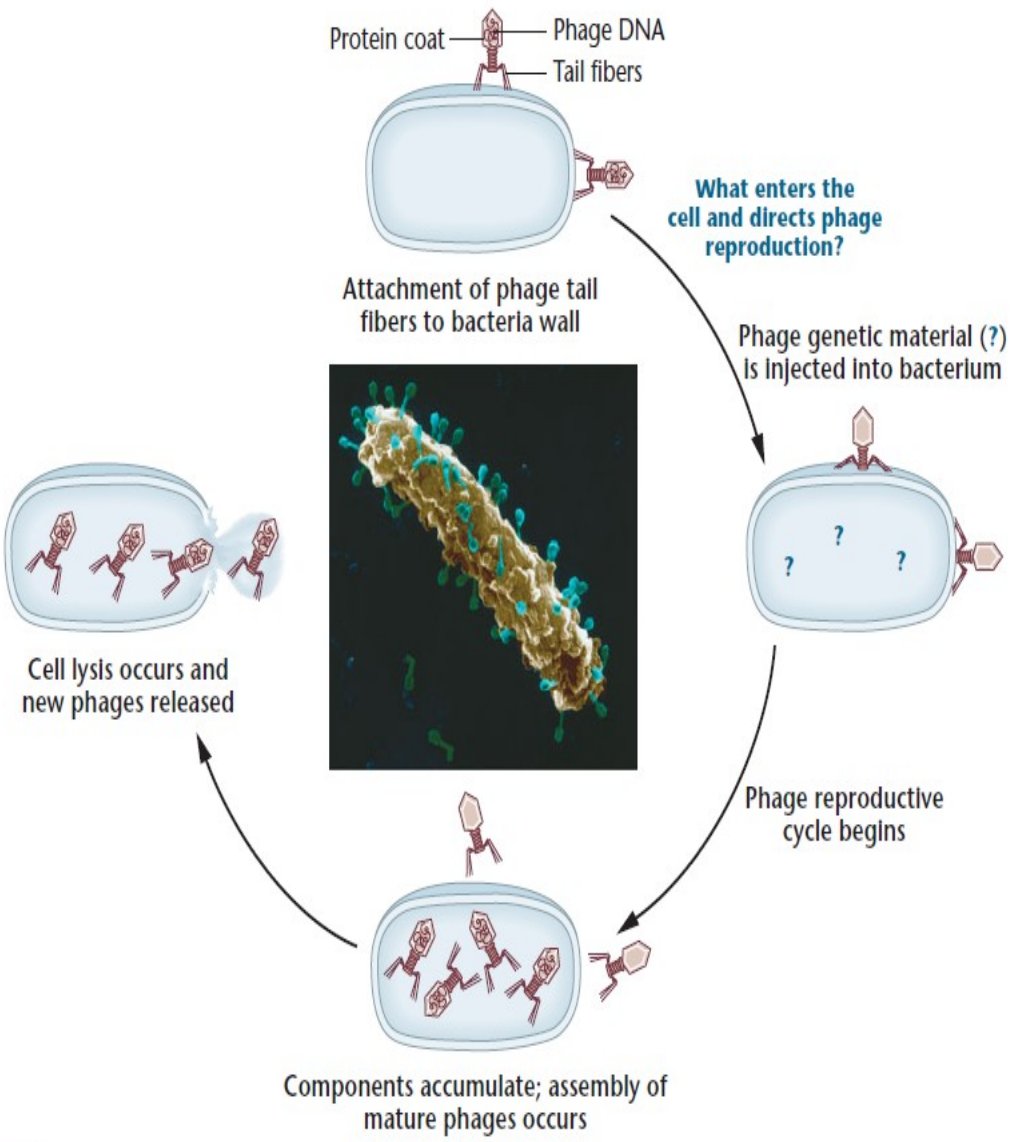

Protein coat
Phage DNA
Tail fibers
What enters the cell and directs phage reproduction?
Attachment of phage tail fibers to bacteria wall
Phage genetic material (?) is injected into bacterium
? ? ?
Cell lysis occurs and new phages released
Phage reproductive cycle begins
Components accumulate; assembly of mature phages occurs

FIGURE 10-4 Life cycle of a T-even bacteriophage, as known in 1952. The electron micrograph shows an *E. coli* cell during infection by numerous T2 phages (shown in blue).

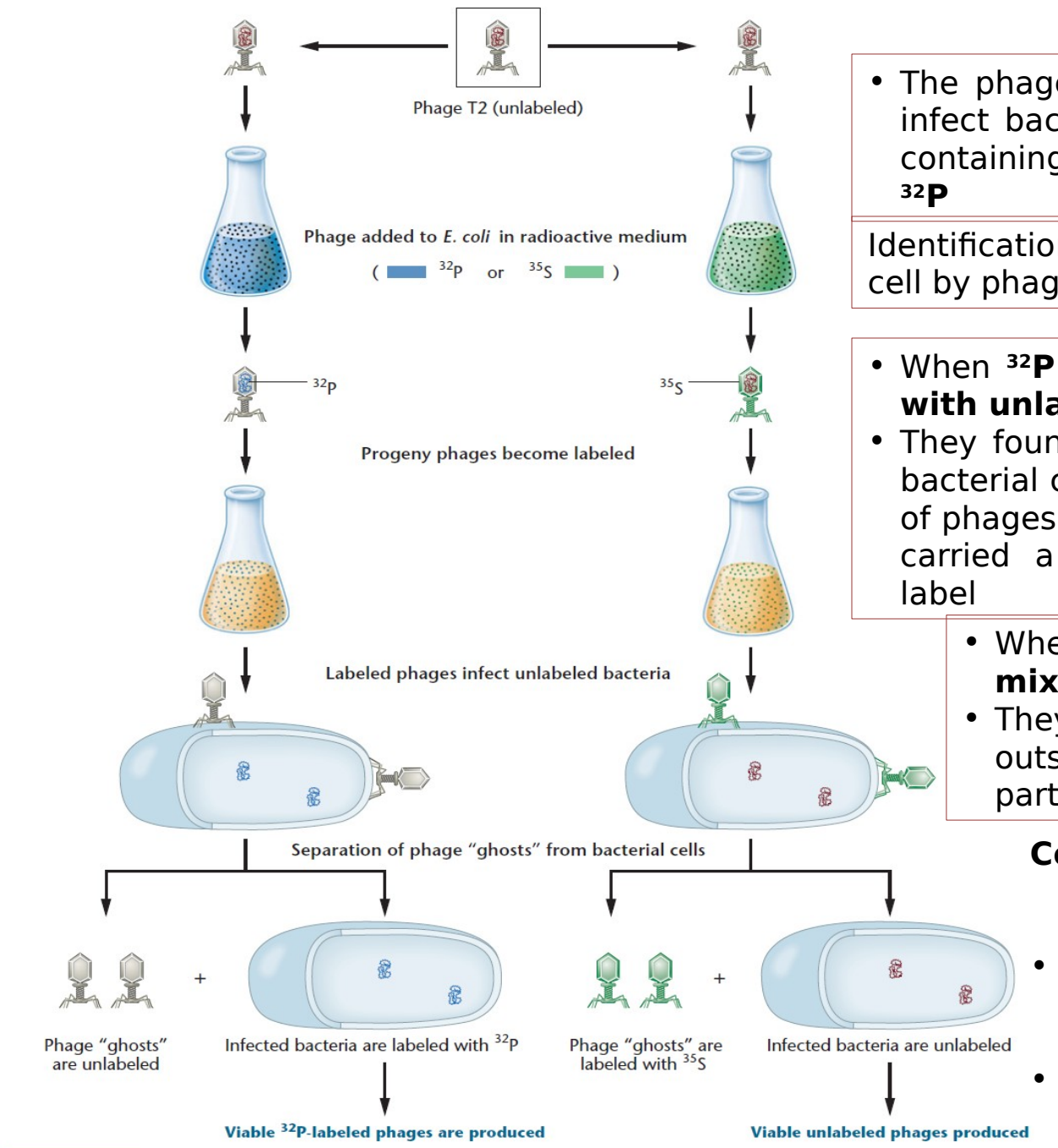

Phage T2 (unlabeled)
Phage added to E. coli in radioactive medium
(32P or 35S)
32P
35S
Progeny phages become labeled
Labeled phages infect unlabeled bacteria
Separation of phage "ghosts" from bacterial cells
Phage "ghosts" are unlabeled
Infected bacteria are labeled with 32P
Phage "ghosts" are labeled with 35S
Infected bacteria are unlabeled
Viable 32P-labeled phages are produced
Viable unlabeled phages produced

FIGURE 10–5 Summary of the Hershey–Chase experiment demonstrating that DNA, and not protein, is responsible for directing the reproduction of phage T2 during the infection of *E. coli*.

- The phages were labeled by having them infect bacteria growing in culture medium containing the radioactive isotopes -**^{35}S** or **^{32}P**

↓

Identification of the material injected into the cell by phages attached to the bacterial wall

↓

- When **^{32}P –labeled phages were mixed with unlabeled *E.coli* cells**,
- They found that the **^{32}P** label entered the bacterial cells and that the next generation of phages that burst from the infected cells carried a significant amount of the **^{32}P** label

↓

- When **^{35}S-labeled phages were mixed with unlabeled *E.coli* cells**,
- They found that the **^{35}S** label stayed outside the bacteria for the most part

Conclusion: The outer protein coat of a phage does not enter the bacterium it infects

- The phage's inner material, consisting of DNA, enter the bacterial cell
- Since **DNA is responsible for the production of new phages during the infection process**, the **DNA**, not the

RNA WORLD HYPOTHESIS

- It proposes that RNA was actually the first life-form on earth, later developing a cell membrane around it and becoming the first prokaryotic cell (the phrase RNA world was first used by Walter Gilbert in 1986)
- This hypothesis is supported by RNA's ability to store, transmit and duplicate genetic information, just like DNA does and to catalyze chemical reactions, just like protein does
- Because RNA can perform the tasks of both genetic materials and enzymes
- RNA is believed to have once been capable of independent life
- RNA- as genetic material
- Some viruses contain an RNA as genetic material
- Experiment that established RNA as genetic material- reconstitution experiment of H Fraenkel Conrat and B Singer

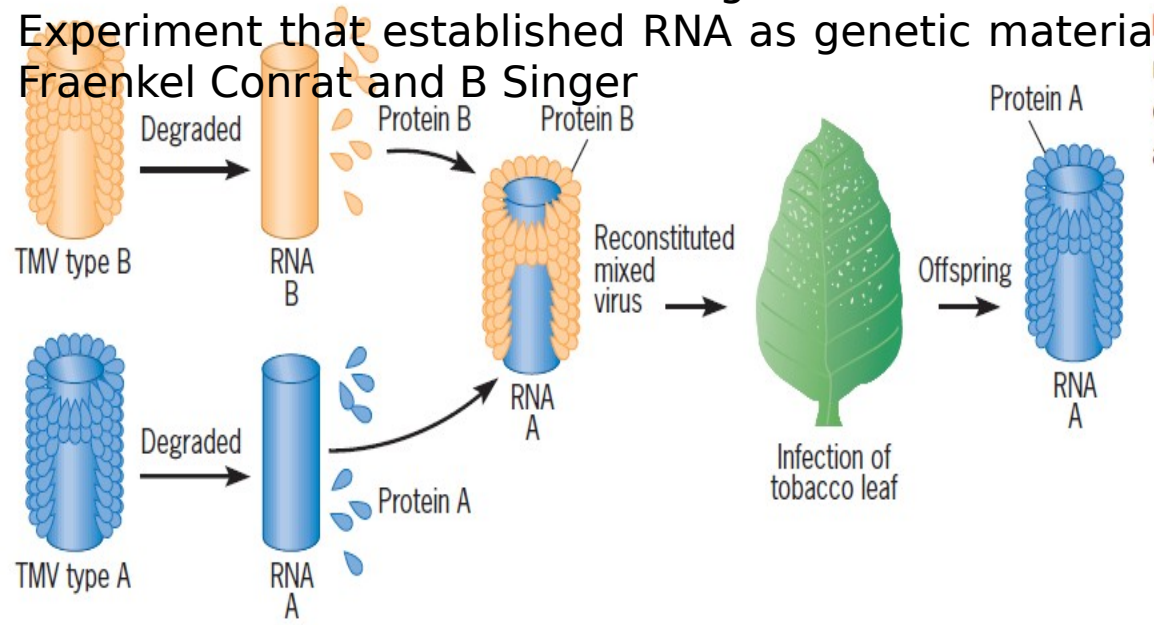
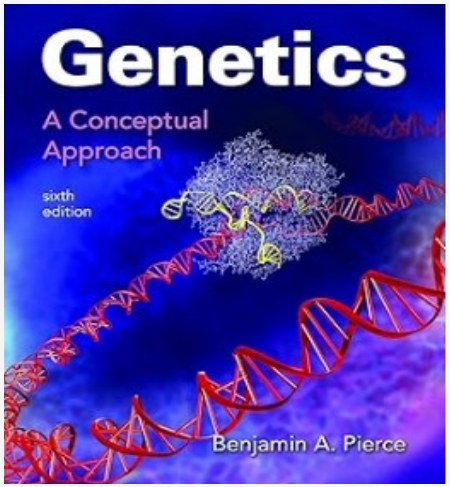


FIGURE 2.4 The genetic material of tobacco mosaic virus (TMV) is RNA, not protein. TMV contains no DNA; it is composed of just RNA and protein.

- 2 different strains of Tobacco Mosaic Virus (TMV)
- Separated the RNAs from their protein coats
- Reconstituted hybrid viruses by mixing the proteins of one strain with the RNA of the second strain and vice versa
- When hybrid virus was spread on tobacco leaves, the lesions that developed corresponded to the TMV from which the RNA had been obtained

REFERENCES: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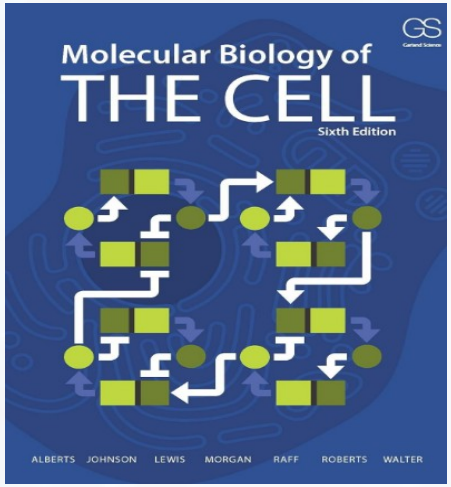
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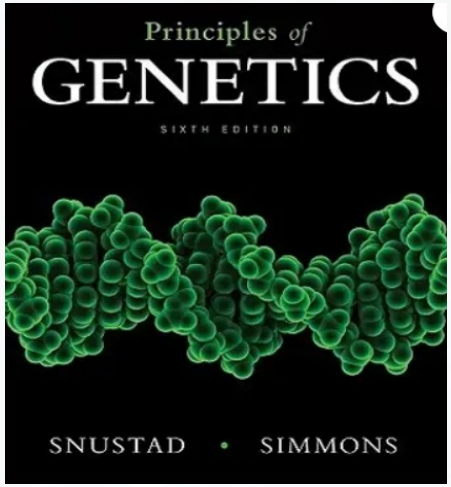
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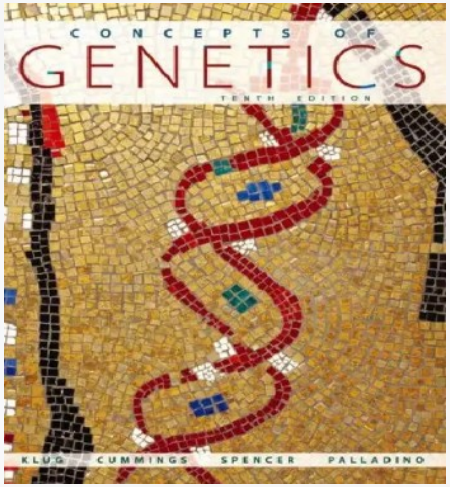
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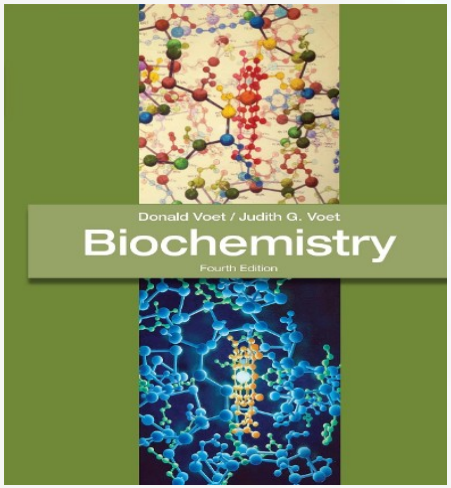
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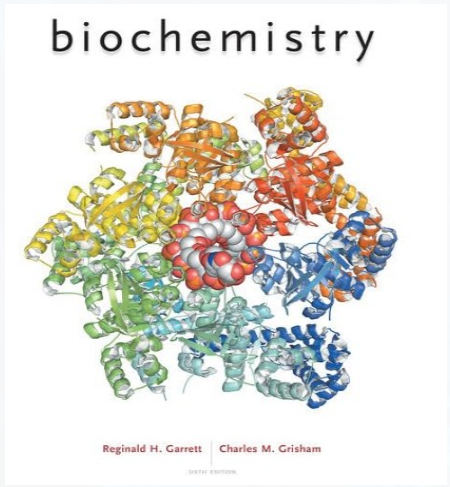
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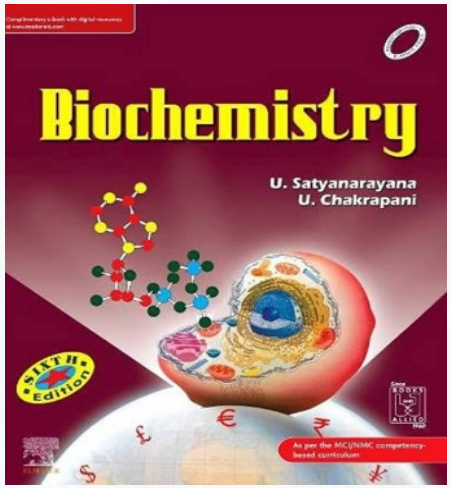
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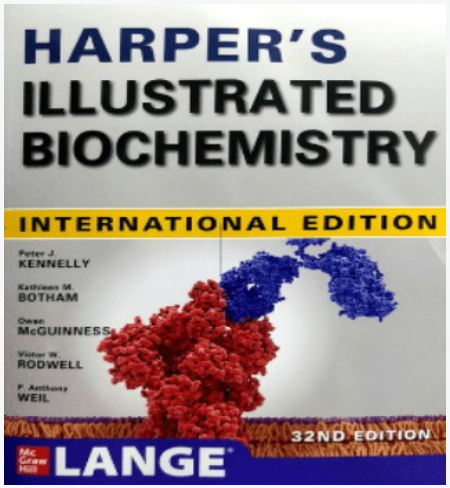
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